Fermentation properties and nutritive value of plantain (*Plantago lanceolata*) silage

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Plantain (*Plantago lanceolata*) is a perennial herb from the plantaginaceae family. The increasing adoption of P. lanceolata by pastoral farmers reflects the growing interest in alternative forages to increase biodiversity and reduce nutrient losses from farm systems. However, adaptation of alternative pasture species requires understanding of how they will respond to typical management practises used for conventional pastures. Conservation of pastures by ensiling is an important forage management practise in any pastoral system. Information is required about the ensiling properties of plantain and subsequent animal response to that product. The objectives of this study, therefore, were to investigate the ensiling properties of plantain, *in vitro* digestibility and in vivo digestibility, and to compare the impact of pre and post ensiling management on plantain ensiling properties. Four experiments were carried out in Canterbury New Zealand between November 2016 - March 2018 :

In the first experiment, the effect of regrowth stage and storage duration on ensiling properties of plantain harvested in late spring were compared using replicated mini-silos (500 g FW) of plantain. Monocultures of plantain were harvested and ensiled at either 4-leaf appearance (4L), 5-leaf appearance (5L) and 6-leaf appearance (6L) and were stored for 90, 120, 150, or 180 days. In spring harvested silage, seed head made up a considerable proportion of the biomass, with increasing seed head numbers from 4L to 6L. This resulted in flow on effects with more fibre and less sugars in the 6L treatment. Pre and post harvest management had significant effects on ensiling properties with later harvesting (6L) having higher pH and lower lactic acid than earlier harvests (4L or 5L). The effect of post harvest storage duration on fermentation characteristics were variable with few consistent trends for pH, fatty acids or ammonia. However, extended storage duration resulted in loss of soluble material increasing overall mean fibre concentrations. Ensiling plantain at earlier regrowth

stage resulted in silages with improved quality characteristics compared with plantain ensiled at 6L. This experiment suggests that silage made from the early regrowth stage and stored as long as 90 days produced the best quality.

In the second experiment, the effect of regrowth stage (4L, 5L and 6L) and storage duration (90, 120, 150, 180 days) on plantain silage produced from autumn was tested. The results obtained were in contrast to the results obtained of experiment 1, because plantain harvested in the late regrowth stage produced silage with improved nutritional characteristics compared with early regrowth. Almost all the variables in fermentation characteristics of plantain 6L were inferior compared with the silage of plantain from the early regrowth stage, though the percentage of mould was the lowest (P=0.01). It is noteworthy that the *in vitro* digestibility of plantain 6L silage was higher than that of silage from the early regrowth stage (76% vs. 71.5%) and also its ME (10.4 MJ/ kg DM vs. 9.3 MJ/kgDM). In this trial, storage duration had no effect on the nutritive value and fermentative characteristics of the silage. This experiment suggests that during autumn, harvesting plantain in the late regrowth stage would be more beneficial than harvesting at an early stage of growth.

In the third experiment, the effectiveness of fertilisers and additives on fermentation and nutritive characteristics were compared for spring harvested plantain at mature harvest (6L) and stored for 180 days. The various fertilisers tested were 20N; 20:1:15 NPK, 40:1:15 NPK; whereas, the additives tested were cellulase, biosil and molasses. The results suggested that there were no interactions between N fertiliser and additive on the fermentation characteristics and nutritive value of the plantain silage. However, the higher N fertiliser resulted in better fermentation characteristics of the plantain silage. Molasses also resulted in better fermentation characteristics for plantain silage, and it decreased NDF, ADF content of plantain silage. Neither the fertiliser nor the additives affected *in vitro* digestibility of the silage.

In the fourth experiment, an *in vivo* DM digestibility study using lambs was conducted to confirm the results from experiments 1 and 3 by using commercially produced baleage silage that had been stored for 90 days. Four treatments: ryegrass silage, plantain silage made from an early harvest (4L), plantain silage made from a late harvest (6L) and plantain silage made from late harvest-treated molasses (plantain 6L+mol), were fed to sheep. Thirty-two male lambs aged 6-12 months with body weights of 36 kg were used for this metabolism stall study. This experiment was conducted in two runs of ten days where each run used 16 animals. Lambs consumed all silages with no evidence that novel plantain silages were less acceptable then conventional ryegrass silage. The results indicated that plantain 4L silage had high *in vivo* DM digestibility compared with other plantain 6L silages, although the digestibility was still inferior compared with ryegrass silage. The *in vivo* DM

digestibility influenced the animal performance where sheep fed plantain 4L silage had better weight gains, although the greatest weight gain was still in sheep fed ryegrass silage. Plantain silage had lower crude protein then ryegrass silage at the same regrowth stage, in spite of similar crude protein at time of harvest. This resulted in lower nitrogen excretion from lambs fed plantain silage ($P<0.01$). The reduction in N excretion could not be explained by plant secondary compounds which were consistently absent from silages throughout all of the experiments.

These studies demonstrated that plantain has some unusual ensiling characteristics in so far as the ensiled plantain does not represent well fermented forage with regards to pH, and low anaerobic stability stability due to high buffering capacity. However it has low butyric acid and in feeding trials animals found it acceptable were able to maintain weight when pre harvest management practises encouraged high nutritive value. Plantain harvested in autumn was more vegetative than that harvested in spring which led to better nutritive value, but vegetative plantain had a high buffering capacity and appeared to have a longer fermentation phase so recommendations for ensiling autumn harvest plantain are to delay harvest to allow maturity and prolong storage to allow fermentation to complete.

Keyword: *Plantago lanceolata* , *Lolium perenne*, ensiling, regrowth stage, fertiliser, additives, digestibility

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List of Abbreviations

Abbreviation	Description	Unit
And	Amylase neutral detergent fiber	% of DM
ADF	Acid detergent fibre	% of DM; g/kg DM
ANOVA	Analysis of variance	
BC	Buffering capacity	meq./100 g DM
BCS	Body condition score	
BIO	Biosil	
CON	Control	
CO_2	Carbondioxide	
CP	Crude protein	% DM
DM	Dry matter	%
DMI	Dry matter intake	kg; %
DOMD	Digestible Organic matter in dry matter	g/kg; %
ENZ	Enzyme	
FW	Fresh weight	
HPLC	High-performance liquid chromatography	
H_2	Hydrogen	
HMW	High molecular weight	
H_2O	Water	
GDD	Growing degree day	
K	Potassium	% of DM; g/kg DM
LMW	Low molecular weight	
LAB	Lactic acid bacteria	
ME	Metabolisable energy	MJ ME/kg DM
MOL	Molasses	
N	Nitrogen	% of DM; g/kg DM
NDF	Neutral Detergent Fibre	% of DM; g/kg DM
NH_3	Ammonia	
NH_3-N	Ammonia Nitrogen	% of total N
Nm	Nano meter	
NO_3	Nitrate	
NPK	Nitrogen phosphorus potassium	
NSC	Non structural carbohydrate	

OM	Organic matter	%
orW	Organic weight	
P	Phosphorus	%
pH	Potential of hydrogen	
Plantain-4L	Plantain four leaves appearance	
Plantain-5L	Plantain five leaves appearance	
Plantain-6L	Plantain six leaves appearance	
rDM	Residue dry matter	
RPM	Rising Plate meter	
rW	Residue weight	
SEM	Standard error of the means	
sW	Sample weight	
VFA	Volatile fatty acid	
WSC	Water Soluble Carbohydrate	%, g/kg DM

Chapter 1

Introduction

1.1. Introduction

New Zealand pastoral contributes 60.4% of New Zealand's agricultural exports (MPI, 2018). Further stated, dairy industry contribution to the gross domestic product increased 13.6% in 2018. Farm systems in New Zealand are based on perennial pasture, which rarely meets its potential animal production but yet is profitable (White, Snow, & King, 2010). New Zealand pasture is dominated by ryegrass (*Lolium perenne* L) and white clover (*Trifolium* repens L) (Zaman, Zaman, Nguyen, Smith, & Nawaz, 2013). Ryegrass pasture, although tolerant of a wide range of grazing and environmental conditions, performs poorly in infertile soils, low rainfall, high soil temperature and is susceptible to attacks by grass grubs and Argentine stem weevils. Furthermore, other grass species are difficult to establish with ryegrass because of its rapid seeding vigour (MacFarlane, 1990).

Recently, more complex pasture mixtures or multi-species pasture have become an alternative to the standard ryegrass and white clover pasture, and they are is characterised by a combination of grasses, legumes and herbs (Daly, Hunter, Green, & Hunt, 1996). One of these herbs is plantain, which is a kind of non-leguminous forb (NLF). Plantain is high yielding and, as the fresh forage demonstrates good quality characteristics (Rumball, 1986). Plantain is palatable and adaptable to grasslands in temperate zones and has the ability to compete with other species in a pasture mixture, although this depends on specific conditions, such as grazing management, fertility levels and pasture species (Stewart, 1996).

Recent research shows increased lamb growth rates on plantain versus conventional pastures (H. Judson, McAnulty, & Sedcole, 2009), and reduced urinary N losses (Totty, Greenwood, Bryant, & Edwards, 2013). Plantain also contains secondary compounds, such as acteoside and catalpol, that are thought to play a role in the reduction of nitrate leaching by reducing nitrification in soils (Carlton, Cameron, Di, Edwards, & Clough, 2019; Woods, Cameron, Edwards, Di, & Clough, 2018) and reduced urinary N losses (R. H. Bryant, Snow, Shorten, & Welten, 2019; Dodd, Dalley, Wims, Elliott, & Griffin, 2019). Because of emerging research showing improved environmental outcomes, there may be increased pressure for farmers to adopt plantain into their system to meet regulatory requirements for lower nutrient losses. However, there remains a paucity of data as to how novel forages might be integrated into farming systems.

The incorporation of plantain into farm systems is expected to yield economic benefits while maintaining or reducing environmental costs. The seasonal growth pattern of plantain is similar to that of perennial ryegrass, which experiences herbage accumulation in excess of animal requirements in spring and summer. Like conventional pastures, plantain reaches peak production in the late spring and summer months, contributing to feed surpluses on farm. Typically, conventional ryegrass pastures are conserved as silage or hay during a feed surplus. Silages, which are typically higher in quality than hays, so are more commonly used, and are based on pastures which are characterised by a high level of rumen degradable protein with a corresponding moderate supply of fermented carbohydrates (Hall & Huntington, 2008). Grass and clover silages preserve relatively well and there is a large body of information describing conditions which ensure optimal silage quality. However, there is no/little published information on the ensilability of plantain – yet farmers anecdotally are already attempting to ensile this forage, with little information to support decision making. Consequently, the purpose of this study was to investigate the ensiling characteristics of plantain forage under a range of management practises and to compare the effect of silage and management on livestock response to plantain silage.

1.2. Aims and objectives

The aim of this study was to determine the effects of management on ensiling of *Plantago lanceolata* on the fermentation characteristics, chemical composition and feeding value of plantain silage.

The specific objectives were :

1. To determine the effect of pre and post harvest management on the fermentation characteristics of ensiled plantain using mini silo's.
2. To determine the effect of pre and post harvest management on the nutritive value of ensiled plantain using mini-silo's.
3. To determine animal response to commercial scale plantain baleage.

1.3. Hypotheses

Under a range of management conditions the ensilability of plantain forage is achievable and the silage produced will have a feeding value equivalent to control silages.

1.4. Thesis structure

This thesis has seven chapters, including this chapter (Introduction). A review of the literature (Chapter 2) provides a summary of the scientific principles of ensiling and evaluates current

understanding of the impact of management of forages on silage quality and feeding value. The objective of chapter 3 was to investigate fermentative characteristics, nutritive value and in vitro digestibility of spring harvested plantain ensiled at different regrowth stage and storage over varying duration. Because plant components differ in different seasons, we conducted a second study in autumn. In Chapter 4, we repeated the spring management practises for autumn harvested plantain silage. In Chapter 5 the objective was to manipulate the quality of the final silage by altering the soil fertility prior to harvest and using additives post -harvest to compare fermentative characteristics, nutritive value and in vitro digestibility of plantain silage. In Chapter 6, we aimed to determine the in vivo feeding value of plantain silage compared with conventional silage using commercially ensiled plantain and ryegrass baleage fed to weaned lambs. Finally, in Chapter 7 we summarise the key findings and implications for further research needed for ensiled plantain.

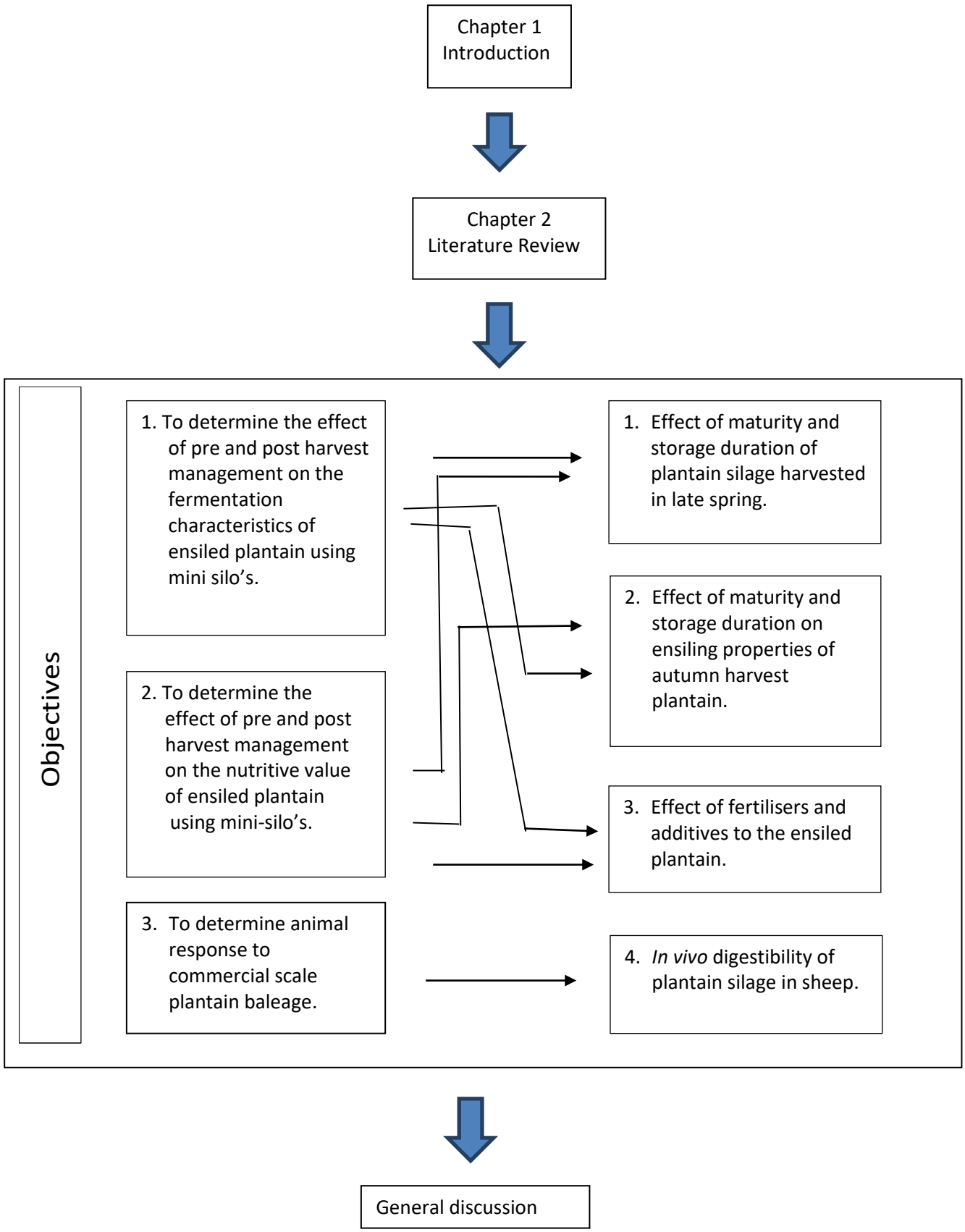

Figure 1.1. Diagram of thesis structure and the relationship among the objectives of thesis.

Chapter 2

Literature review

2.1 Pastoral systems in temperate countries

2.1.1 Balancing feed supply and demand

In all temperate pastoral-based agriculture, there is a positive relationship between the amount of pasture grown per hectare and the operating profit of livestock systems from grazing livestock (Chapman, Kenny, Beca, & Johnson, 2008; Savage & Lewis, 2005). For the dairy industry in New Zealand and Australia, the efficiency of the grazing system contributes significantly to the cost competitiveness (Chapman, Rawnsley, Cullen, & Clark, 2013). However, one of the dominant causes of inefficiency in pasture-based livestock production is the variability in pasture growth over the seasons. Grass dominant pasture in New Zealand covers seven million hectares that carries 60 million sheep and cattle (Siegel, Latch, & Johnson, 1985). Pasture for grazing in New Zealand is dominated by ryegrass (*Lolium perenne* L) with some white clover (*Trifolium repens* L) (Woodward, Waugh, Roach, Fynn, & Phillips, 2013; Zaman *et al*. 2013). This binary combination of pasture species is a popular choice because it is high yielding over a long season, aligning with the nutritional demands of spring-calving animals. The goal for farmers to maintain pasture quality is to control post grazing residuals to encourage regeneration of high-quality leaf and to avoid accumulation of stem and dead material which are more difficult to digest. For controlling post-grazing residual sward height, rotation length, and the daily allocation of pasture area to animals, pastures are grazed *in situ* throughout the year (MacDonald, Glassey, & Rawnsley, 2010). If there is too much pasture for animals to consume, the surplus pasture can be conserved as silage or hay.

Effective grazing management practises will try and find a balance between feed supply and feed demand of animals. Where feed supply refers to the total amount of nutrients/energy available from pasture and feed demand refers to the total amount of nutrient/energy required by grazing livestock for maintenance and production (Chapman *et al*. 2013). Sustainability and productivity of ruminant livestock systems can be improved by better management of the feed base. In a temperate country, the feed demand exceeds feed supply during winter and early spring but, provided a farm is not overstocked and feed surpluses can be conserved, sufficient feed is carried over from the summer (Bell, Robertson, Revell, Lilley, & Moore, 2008). Further stated, during winter (May-August) it is common to have insufficient feed supply. Thus, during this season, farmers usually provide feed supplements to their animals, or reduce animal demand by selling stock or wean/dry-off earlier, to

support the animals during winter. Other management decisions that can overcome the feed deficits at different times of year is increasing or decreasing inputs of nitrogen fertiliser, irrigation water, altering the frequency and/or severity of grazing, culling cows early or conserving excess feed as silage or hay (Chapman *et al*. 2013).

2.1.2 Conservation of feed supply during a feed surplus

In many countries, including New Zealand, when there is an abundance of forage, it is often preserved as silage. The purpose of storage systems is to preserve the quality of feeding characteristics as closely to that of the fresh form, and that dry matter (DM) and energy losses should be minimized (Muck, 1988; Van Soest, 1983). Commonly, there are two forms of conserved forage: namely, silage and hay. Research conducted by Sormunen-Cristian & Jauhiainen (2001) found that grass hay had a lower digestibility, contained less protein and had higher fibrous constituents than silage. The quality characteristics differ between hay and silage as a result of the maturity at which they are harvested. With silage being a 'wet' feed, the original forage can be harvested whilst still green and leafy. On the other hand, hay is harvested when the forage reaches advance maturity, usually during reproductive development to encourage high yields. For silage, factors affecting silage quality are largely influenced by the parent material and how the pasture was managed prior to time of harvest. Pasture management can alter the 'ingredients' for good fermentation, while the extent of wilting and use of additives can aid fermentation.

2.2 The ensiling process

The principles of ensiling are to achieve acidic, anaerobic conditions to discourage the growth of undesirable microorganisms (Meeske, 2005). Effective ensiling has been described as taking place over four distinct phases: 1. Aerobic phase, 2. Fermentation phase, 3. Stable phase and 4. Feed out phase (Weinberg & Muck, 1996). Figure 2.1. demonstrates the various phases of silage fermentation. This section will describe what happens in those phases and outline the environment, plant and management factors which can influence each of the processes which take place during these phases.

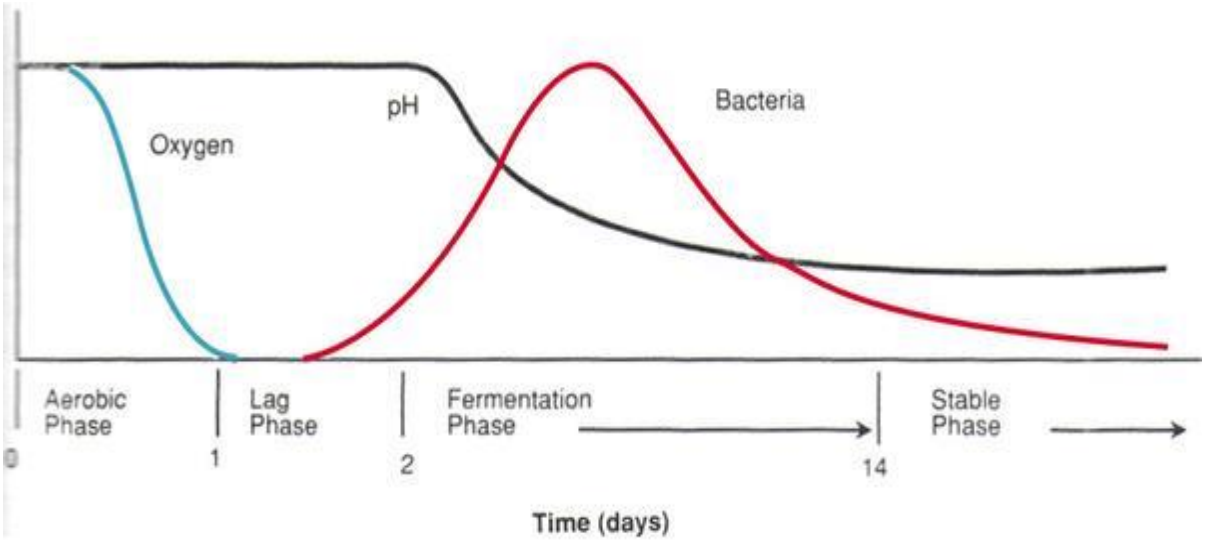

Figure 2.1. The phases of silage fermentation (sourced from https://content.ces.ncsu.edu/forage-conservation-techniques-silage-and-haylage-production).

2.2.1 Aerobic phase and lag phase

Aerobic phase lasts for several hours generally at harvesting temperature of around 20°C. During this phase the pH is in the normal range, which is between 6 - 6.5 (Weinberg & Muck, 1996) and oxygen is still present between plant particles. This condition allows plant respiration, enzymes and aerobic and facultative aerobic bacteria such as yeast, fungi and enterobacteria still to be active that generate heat. Within the first days of ensiling, oxygen is depleted, and lactic acid bacteria populations are still low, so this is regarded as the lag phase with little shift in pH during this period. Proteases decompose protein to amino acids and carbohydrases increase the amount of soluble carbohydrates available for fermentation. A fine chopped, well-compacted silage crop and good silo, filling technique in this phase could reduce the negative impact of oxygen (Mc Donald, Henderson,& Heron, 1991).

2.2.2 Fermentation phase

The fermentation phase commences once anaerobic conditions have been reached. The main fermentation phase continues for a week but it can last for more than a month, depending on crop properties and ensiling condition (Pahlow, Muck, Driehuis, Elferink, Spoelstra, 2003). Microorganisms play an essential role during the fermentation phase as they are responsible for producing lactic acid and reducing the pH of the silage, that decreases to 3.8 - 5.0 (Weinberg & Muck, 1996). The first stage of fermentation is the death of plant tissue and the rapid depletion of

oxygen. This is then followed by the development of lactic acid fermentation and a reduction of pH through acid production (Watson & Nash, 1960). Plant respiration, anaerobic fermentation, aerobic decomposition, especially at surfaces, and effluent loss cause feed energy losses during ensiling. (Van Soest, 1983). Rapid exclusion of air promoting an anaerobic environment is critical to minimizing losses during storage and feeding out (Woolford, 1990). Anaerobic conditions can be achieved by wilting the forage before ensiling, consolidating and compacting the material and sealing the silo (Mc Donald, Henderson, & Heron, 1991). Anaerobic condition supports primary fermentation that produces lactic acid. However, if this condition is not met, then secondary fermentation will have occurred. Residual plant sugar and lactate are fermented to butyric acid by which pH increases and amino acids are fermented to amines and ammonia (NH_3) (Pahlow *et al.* 2003).

2.2.3 Stable phase

During the stable phase few changes occur in the silo if anaerobic condition can be maintained. Highly acid tolerant yeast become inactive and bacilli and clostridia remain in a state of dormancy as endospore. Only acid tolerant enzymes of proteases, carbohydrases, some specialized microorganisms for example *lactobacillus buchneri are* still active. Acid tolerant enzymes cause a slow acid hydrolysis of structural and storage carbohydrate. Proteases convert Nitrogen (N) compounds to Ammonia. In silage crop with sufficient water-soluble carbohydrate (WSC) the stable phase can be any length. However, practically it does not exceed the following harvest season (Pahlow *et al.* 2003).

2.2.4 Feed out phase

This phase allows oxygen to re-enter the silo, and aerobic spoilage is started because aerobic microorganisms such as yeast, mould, bacilli and acetic acid bacteria become active again. During the feedout phase, the DM losses can be around 0.35 – 2.7% per day (Woolford, 1990). This spoilage can be minimized by adopting a high feed out rate (i.e. emptying the silo quickly) and an airtight silo as with baleage.

2.3 Silage microflora

There are two types of microflora that play an important role in the ensiling process, and during fermentation as well as feed-out i.e. in aerobic phase. Microflora can be categorized into two

groups namely desirable and undesirable microflora (Elferink, Driehuis, Gottschal, & Spoelstra, 2000). Lactic acid bacteria (LAB) form the basis of the desirable microflora. Whereas microflora that causes anaerobic (e.g. Clostridia and enterobacteria) or aerobic deterioration (such as yeast, bacilli, listeria and mould) are categorized as undesirable microflora.

The source of bacteria which are essential for the fermentation process are present on the fresh forage but in relatively low populations. The population distribution of microflora in fresh forage are different from microbes in the fermentation process. The list of microflora on fresh grass and herbage are summarised in Table 2.1. Many species are classified as aerobic bacteria and these are usually replaced by anaerobes when the process of silage fermentation started (Van Soest, 1983).

Table 2.1. Microflora population on plants prior to ensiling (Pahlow *et al*. 2003).

Group	Population (CFU/g crop)
Total aerobic bacteria	>10.000.000
Lactic acid bacteria	10- 1.000.000
Enterobacteria	1.000 – 1.000.000
Yeast and yeast like fungi	1.000 – 100.000
Moulds	1000 – 10.000
Clostridia (endospores)	100 - 1000
Bacilli (endospores)	100 – 1.000
Acetic acid bacteria	100 – 1.000
Propionic acid bacteria	10 - 100

Lactic Acid Bacteria

Lactic acid bacteria (LAB) are the most important microflora for silage fermentation. The genus of lactic acid bacteria are *Lactobacillus, Pediococcus, Lactococcus, Enterococcus, Streptococcus, Leoconostoc* and they produce lactic acid as the principal product from fermenting sugar, acetic acid, ethanol and carbondioxide (CO_2) (Pahlow *et al*. 2003). According to Ohmoto, Tanaka, Kitamoto, & Cai (2002), *Lactobacillus* species, such as *Lactocooccus lactis, Enterococcus faecalis, Pediococcus acidilactici, Leuconostoc mesenteroides, Lactobacillus plantarum* and *Lactobacillus cellobioses*, grow together in the early fermentation stage with yeasts and moulds, due to the presence of air between the plant particles. At the end of the fermentation stage, *Lactobacillus* sp. becomes prevalent, due to their tolerance to acid conditions. Silage LAB are quite well diversified, and dominance of the different LAB species depends on the properties of the plant material properties, silage technology

and silo type. For example, in good silage of orchardgrass and lucerne, the fermentation is initiated by streptococci, latter supported by the pediococci and leuconostocs and completed by lactobacilli (Langston, Bouma, & Conner, 1962). LAB can reach in peak number of 10^{10} CFU g^{-1} (Pahlow *et al*. 2003).

Enterobacteria

Enterobacteria are the second largest population of microflora in silage. Most enterobacteria in silage are non-pathogenic and facultatively anaerobic (Woolford, 1984). These microflorae are not desirable because they compete with LAB for WSC and degrade protein. The degradation of protein reduces the feeding value and may lead to the production of toxic compounds such as biogenic amines and branched fatty acid. Biogenic amines have negative effect on silage palatability. In spite of the depletory effects of enterobacteria there is a small, yet significant role of this microflora in reducing Nitrate (NO_3), resulting in the production of nitrite and nitrogen oxide gases which inhibit clostridia growth (Pahlow *et al*. 2003). Some of the enterobacteria present in silage are *Hafnia alvei*, *Escherichia coli*, *Serratia fonticuli*, *Enterobactercloacea*, *Citrobacter spp*. (McDonald *et al*. 1991).

Clostridia

Clostridia are endospore-forming anaerobic bacteria. The signs of clostridial silage are high butyric acid content of more than 5g/kg DM, a high pH (>5 in low DM silage), high ammonia and amine content (Macpherson & Violante, 1966). This microflora can be dangerous because clostridia spore can survive passage through the digestive tract of dairy cattle. Clostridia can contaminate milk via faeces, and faecal contamination of the udder. The more common species is *Clostridium tyrobutyricum* which degrade lactic acid to butyric acid with the following reaction:

$$2 \text{ lactic acid} \rightarrow 1 \text{ butyric acid} + 2H_2 + 2CO_2$$

Clostridium botulinum is extremely toxic that can be deadly for cattle. However, *Clostridium botulinum* is not able to grow in well fermented silage.

Yeasts and moulds

Yeasts generally initiate aerobic deterioration. Yeasts consume sugar and fermentation acids. These microorganisms raise the pH and the temperature of the silage (Pahlow *et al*. 2003). During the first weeks of ensiling, the population of yeast can reach up to 10^7 cfu/g crop, but they decrease gradually during prolonged storage. Some of yeast found in silage are *Candida, Hansenula, Saccharomyces* and *Torulopsis* (McDonald *et al*. 1991). Increase in pH causes bacilli and other aerobic bacteria to develop and subsequently causes an increase in temperature. Finally, with an

increase in pH, moulds grow well and deteriorate the silage. Moulds are aerobic microorganisms that are slow growers. Moulds are important because they produce mycotoxins that will affect animal health (Muck, 2010). Woolford (1984) reported that moulds in silage belong to the genera *Penicillium, Fusarium, Aspergillus, Mucor, Byssochlamys, Absidia, Arthrinium, Geothricum, Monascus, Scopulariopsis* and *Thrichoderma*.

Bacilli

Bacilli are endospore forming rod shaped bacteria which are (facultative) aerobes. Bacilli can enhance aerobic deterioration (Lindgren, Pettersson, Kaspersson, Jonsson, & Lingvall, 1985). Bacilli spores can be transferred to milk via faeces. Storage temperatures should be low to reduce bacilli growth (Gibson, Stirling, Keddie, & Rosenberger, 1958). To minimize bacilli infestation, contamination of the herbage material with soil or manure at time of harvest should be prevented. Some bacilli species that are usually found in silage are *Bacillus coagulans, Bacillus licheniformis* and *Bacilli polymiyxa* (McDonald *et al*. 1991).

Acetic acid and propionic acid bacteria

Acetic acid and propionic acid bacteria are less important in ensiling process. Propionic acid bacteria may affect the fermentation and storage process (Pahlow & Honig, 1994). Propionic acid bacteria such as *Propioni bacteria* ferment lactate to propionate in low pH silages. Propionic acid inhibits the growth of mould at low pH (McDonald *et al*. 1991). However, acetic acid bacteria are most important for maize silage as acetic acid bacteria alone can initiate aerobic deterioration (Spoelstra, Courtin, & Van Beers, 1988). (Muck, 2010)stated that acetic acid bacteria are classified as aerobic bacteria that are able to live at low pH. They can grow on ethanol, producing acetic acid + CO_2 + H_2O. Furthermore, they raise the pH and permit other aerobic microorganisms to grow. Acetic acid bacteria belong to the genus *Acetobacter* that is divided in the subgenus *Acetobacter* and *Gluconoacetobacter* (McDonald *et al*. 1991).

2.4 Silage characteristics

Characterising silage quality can be defined as the sum of two parts. Firstly, the silage quality can be defined by the quality of the fermentation characteristics and secondly, by the nutritional value of the product itself, both of which are important parameters for animal response to silage feeding and livestock production.

2.4.1 Fermentation characteristics

Moran (2005) stated that unsatisfactory silage has a very unpleasant and strong smell of ammonia, a high moisture content, is mouldy, has much deterioration, and is slightly damp and dark brown. The colour of the silage is generally the result of aerobic conditions with oxygen allowing respiration to continue for long periods. One of the criteria for determining good fermentation characteristics of silage is pH, where pH is a measure of acidity (Kung Jr, Shaver, Grant, & Schmidt, 2018).

The pH of silage is very important for preventing growth of undesirable bacteria and yeasts. If dry matter content of the original forage is low, a rapid decline in pH becomes crucial (Muck, 1988). Kung & Shaver (2001) reported that the pH required for grass silage stability at dry matter contents of 25% -35% is 4.3 - 4.7. Muck & Shinners (2001) stated that ensiling with a 30-50% DM gives the best result as it becomes increasingly difficult to reach target low pH in high moisture forages. The effect of moisture on pH requirements highlight the need for fast and effective wilting of the forage/crop prior to ensiling. Kung & Shaver (2001) set targets for good fermentation characteristics of silage as listed in Table 2.2.

Another factor influencing silage pH and the rate of decline of pH is the buffering capacity (BC) of the forage, but little has been published about buffering capacity and the factors affecting its variability (Muck, O'Kiely, & Wilson, 1991). Greenhill (1964) defined buffering capacity of herbage is its ability to resist pH change as the result of the addition of acid or alkali. Thus, buffering capacity is measured as the equivalent amount of acid per unit DM required to lower crop pH from 6 to 4. Legumes have the highest BC followed by grasses and whole plant maize (Muck *et al*. 1991). The lower the BC the better, as this is indicative of the crop to easily ferment i.e. achieve the desired low pH with less lactic acid. The BC of good grass silage is 1.120 meq kg^{-1} DM (McDonald *et al*. 1991). Most of the herbage BC is associated with the anion fraction such as organic acid salts, sulphates, orthophosphates, nitrates and chlorides (Playne & McDonald, 1966). For instance, anion fractions contribute 68% of the total herbage BC and about 80% of the silages. On the other hand, proteins make a small contribution to the BC of herbage. During ensiling, BC increases markedly because of the production of lactic acid and acetic acid.

Table 2.2. Targets for high quality and well preserved silage(adapted from Kung & Shaver, 2001).

Item	Legume silage <30-35% DM	Legume silage 45 – 55% DM	Grass silage 25 – 35% DM
pH	4.3 – 4.5	4.7 – 5.0	4.3 – 4.7
Lactic acid , %	6 – 8	2 – 4	6 -10
Acetic acid, %	2 – 3	0.5 – 2.0	1 – 3
Propionic acid, %	<0.5	<0.1	<0.1
Butyric acid, %	<0.5	0	<0.5 – 1.0
Ethanol, %	0.5 – 1.0	0.5	0.5 – 1.0
NH_3-N, % of total N	10 – 15	<12	8 – 12

2.4.2 Nutritional characteristics

The aim for silage production is to try and ensure the nutritional characteristics of silage do not differ greatly from the fresh form (Van Soest, 1983). On the basis of nutritional availability, forage dry matter can be divided into two fractions. The first fraction is the cellular content that is composed of lipids, soluble carbohydrates, protein and other water-soluble matter. The second fraction is plant cell walls, which comprise hemicellulose (which is a large fraction), cellulose and lignin together. The nutritive availability of the cell wall is different among different forages (Van Soest, 1967). Some authors have given recommendations for what constitutes good quality silage (Table 2.3), and these values require tools which can provide this information for quantifying the nutritional value of silage. Near Infrared Reflectance Spectroscopy or NIRS has become popular as NIRS provides rapid, comprehensive and inexpensive results (Offer, Percival, Dewhurst, & Thomas, 1998). NIRS relies on the measurement of light absorption using wavelengths in the infrared region of the spectrum of 1100-2500 nm (Beever & Mould, 2000). The results of the absorption spectrum are influenced by the chemical bonds within the constituents of the sample so, therefore, it is possible to identify the specific region of the spectrum associated with different chemical entities, such as protein, fibre, starch, etc. However, calibration of the NIRS apparatus across standard reference samples that have been analysed by routine 'wet chemistry' methods is needed first to have confidence in these tools.

Whether measured using wet chemistry or NIRS methods, forage fibre analysis is an important measure because forage fibre concentration is closely correlated with digestibility (Beever & Mould, 2000). In maize silage for example, the target for good silage would contain an NDF of 413 g kg^{-1} DM and an ADF of 219 g kg^{-1} DM (McDonald *et al*. 1991). Regarding the protein content, protein is crucial for silage because it affect animal performance and environment. Strategy to reduce N losses and improve N efficiency should focus on protein that contain nitrogen should focus on an optimal

supply of rumen degradable N and optimal efficiency of utilization of absorbed amino acid s for production. N losses should be considered as well (Djikstra *et al*. 2013).

In terms of the digestibility of silage, the digestibility of any feed given to livestock has implications on animals' intake and subsequent performance. As with fibre content, digestibility can be determined by in vitro methods such as wet chemistry or NIRS or using *in vivo* techniques. Good silage should have a digestibility of more than 70 % to reduce intake limitations (Table 2.3). *In vivo* digestibility is defined as the apparent digestibility of the feed, where the part of the feed that is not excreted in the faeces is assumed to have been absorbed by the animal (McDonald, Edwards, Greenhalgh, & Morgan, 1995). However, i*n vivo* digestibility can be estimated by using *in vitro* digestibility procedures that are cheaper and more convenient (Aregheore, 2000). Furthermore, *in vitro* techniques have become an important tool for ruminant feed evaluation (Menke *et al*. 1979). Van Soest (1983) stated that nutritive values derived from i*n vitro* digestibility should only be treated as estimates. However, Pirie (1987) opined that if a feed nutrient is readily digested by several enzymes *in vitro*, it will be digested *in vivo* as a result of the simultaneous actions of several enzymes and with possible cooperation from the rumen flora. *In vitro* digestibility using pepsin-cellulase technique can be used to estimate *in vivo* dry matter digestibility and organic matter digestibility with high accuracy (De Marco, Peiretti, Miraglia, & Bergero, 2014)

Factors contributing to lower *in vitro* digestibility include buffer pH, the base diet of the animal from which rumen fluid is supplied and the quality of feed samples taken for testing. Burbank, Woolf, & Post (1979) reported that the *in vitro* digestibility was lower in a system buffered at pH 5.6 compared to pH 7.0. The variation in pH of the rumen fluid and the extent of fermentation of the samples is governed by the chemical composition of the food, especially carbohydrates and this makes it difficult to incubate the sample at an optimum pH throughout the process. Because of the variation in rumen fluid from donor animals, *in vitro* digestibility techniques were established using enzymes in order to improve repeatability. Insignificant differences in the *in vitro* and *in vivo* digestibility relationships for grasses, legume, and hay were found by Alexander & McGowan (1966). The pepsin cellulose method was more accurate for the organic matter digestibility (OMD) of silage than for hay (Forejtová *et al*. 2005).

Table 2.3. Targets for high quality and well preserved silage (adapted from Howse *et al*. (1996)

Factor measured	Target for high quality silage
DM % (pit or stack silage)	25 – 30
DM % (baled silage)	30 – 35
Crude protein	16 – 20 %
Digestibility	> 70%
Metabolisable energy	> 10 MJ ME/kg DM

2.5 Factors affecting silage characteristics

There are several environmental, plant and management factors and their interactions will contribute to the success of the ensiling process. For example, choice of crop and harvest maturity can influence the moisture content and the sugar content of the plant and flow on effects on substrate availability during the fermentation phase. This section will explore the factors affecting silage characteristics.

2.5.1 Environment

Season

Season is important in influencing silage quality. Season and geographical location affects forage quality (Buxton, 1996). The time of the previous harvest, annual and seasonal variations in temperature, radiation, water availability, etc., contribute to changes in the type of regrowth (vegetative vs generative) and rate of cell wall lignification (Van Soest, 1983). The DM percentage and harvest season may affect the intake characteristics of silage (Kuoppala, Rinne, Ahvenjarvi, Nousiainen, & Huhtanen, 2004). Moreover, the chemical composition of silage is influenced by the season (Seng, Bonorden, Nissen, Isselstein, & Abel, 2008). Table 2.4. shows that spring cut silage had lower DM, pH, crude protein than those for silage cut in summer.

Table 2.4. Dry matter(DM) content, fermentation characteristics and chemical composition of ryegrass silages when fertilised with nitrogen (GN), when grown with clover (GC) or with a mix of plant species (GCF) (adapted from Seng *et al*. 2008).

	Spring			Summer		
	GN	GC	GCF	GN	GC	GCF
DM of silage (g/kg)	218	219	190	229	242	228
pH	4.3	4.2	4.9	5.7	4.9	4.7
Constituents (g/kg DM)						
- Organic matter	913	918	905	894	890	897
- Crude protein	227	166	146	225	184	154
- Ether extract	42	44	41	34	37	30
- Crude fibre	461	426	346	521	440	390
- Non-fibre carbohydrates	183	282	372	114	228	323

Furthermore, (Buxton, 1996) stated that a rise in air temperature in which forages are grown usually increases the rate of plant development, stem/leaf ratios but decreases the digestibility. Thus, increasing temperature results in a lower forage quality even when compared at the same morphological stage. A 1°C increase in temperature will generally decrease digestibility of cool-season forages by 3-7 g/kg. with only minor effects on crude protein concentration (Wilson & Minson, 1983). This is one reason why forages produced at high elevations with their lower temperatures tend to be of a higher quality than those produced at low elevations.

2.5.2 Plant factors

Moisture content

For ensiled forages, post-harvest procedures with respect to field wilting, additive applications, and ensiling conditions are important (Aregheore, 2000). Pitt, Muck, and Leibensperger (1985) found that the plant respiration rate, plant enzyme activities, and microbial growth rates could be reduced by the increasing DM content. This is important as the respiration of plants or microbial activity removes the digestible components of the crop and this is the cause of much of the DM loss during silo storage (Muck & Shinners, 2001). Van Soest (1983) suggested that the moisture content of silage should be kept above 45% to limit heating and Maillard reactions. Moisture is also of great importance for speed of fermentation i.e. drier silages ferment slower and have less acids and higher pH, e.g. in previous studies the pH decreased more slowly in silage with 54% DM compared with silage with 30% DM content (Whiter & Kung Jr, 2001).

The influence of DM levels on proteolysis in silage has shown that increased dry matter resulted in decreasing proteolysis in corn (Lopez, Jorgensen, Larsen, & Niedermeier, 1970), alfalfa (Merchen & Satter, 1983) and ryegrass (Henderson, McDonald, & Anderson, 1982). Proteolysis resulted in nitrogen transformation from protein to non-protein N and this transformation is essential because the quality of crude protein in silage may affect its utilization by the animal (Wilkins, Hutchinson, Wilson, & Harris, 1971). In silage, most of the nitrogen transformation is the result of either plant enzymes or microbial degradation.

Sugar and nitrogen content

Fermentation bacteria need various nutrients for growth. Within a short time, the dominant bacterial population in silage should be lactic acid bacteria (LAB) due to their important role in producing lactic acid and reducing pH (Section 2.2.2). To support the growth and activity of LAB, the forage should contain adequate sugars, complex N sources, such as amino acids, purines and pyrimidines, and vitamins (Pahlow & Honig, 1994).

Sugar is used by LAB as an energy source (Gibbs, Dumrose, Bennett, & Bubeck, 1950) as after hydrolysis of sucrose, the remaining glucose and fructose supply the carbon needed by LAB. Haigh & Parker (1985) reported that a minimum required WSC for successful ensiling in fresh herbage is 25-30 g kg^{-1}. However Haigh (1990) later found that the minimum WSC required in herbage with a DM of 230 g kg^{-1} was 37 g kg^{-1}. In a study comparing fermentation characteristics of different grass species harvested at differing maturities when grown under high and low N fertiliser. King, McEniry, Richardson, and O'Kiely (2013) showed a negative relationship between WSC and pH up to a maximum WSC content of about 20 g kg^{-1} DM after which no further reductions in pH were observed. When sugar levels were less than 20 g kg^{-1}, pH could be reduced by 0.05 for every unit increase in soluble sugar content (Figure 2.2).

Water soluble carbohydrates (WSC) play an essential role in ensiling as they are used by the microbial population to produce lactic acid, and reduce pH (McDonald *et al*. 1991). The relationship between plant sugars and pH have been reported by (King *et al.* 2013) that can be seen in Figure 2.2.

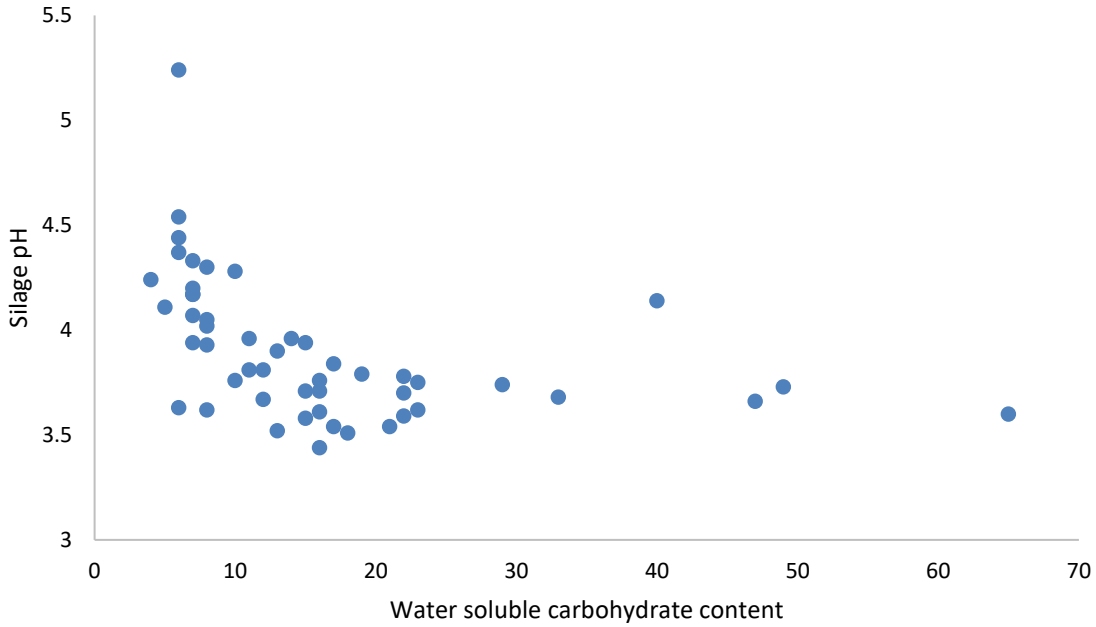

Figure 2.2. Relationship between pH and WSC in silages made from different grass species at different maturities and N fertiliser level. Adapted from King *et al*. (2013).

Forage type

The type of forage influences the quality of the silage. Grass is easier to ensile than legumes, for instance, due to its low buffering capacity enabling fermentation to low pH (Heinritz, Martens, Avila, & Hoedtke, 2012). The difficulty of ensiling legumes has been attributed to their high buffering capacity, high protein content (McDonald & Henderson, 1962), and low WSC concentration (Dewhurst, Fisher, Tweed, & Wilkins, 2003) and additional risk of butyric acid fermentation (Hattori, Kumai, & Fukumi, 1996). However, legumes also contain many buffers, such as phosphates, carbonates, malates, oxalates, tartrates, asparagine, potassium, calcium and magnesium which do enable them to be ensiled (McDonald & Henderson, 1962).

Legume grains, such as lupin, field beans and peas are valuable feedstuffs particularly because of their contents of protein and essential amino acids. These grains, however, mature at different rates even on the same field and, furthermore, they contain several anti-nutritional factors, such as oligomeric carbohydrates, tannins and alkaloids. Ensiling moist-harvested legume grains mitigates the problem of inconsistent maturity and, furthermore, microbial fermentation may decrease the amount of some anti-nutritional factors (Zeyner, Gefrom, Hillegeist, Sommer, & Greef, 2015). In spite of the difficulty ensiling legumes, they often have better acceptability by the animal than grass silages (Weiss, 1995).

Plant Secondary metabolites

A huge variety of chemical compounds or secondary metabolites exist in vascular plants which make a major contribution to the specific odour, tastes and colours of plants (Bennett & Wallsgrove, 1994). Many secondary metabolites are key components of active and potent defence mechanisms of plants to aid defence against pests and pathogens. Many compounds are synthesized by and accumulate in young developing tissue, especially in leaves, or in reproductive tissue such as flowers and seeds. While secondary metabolites are important as protection from harmful environmental conditions, they can also be restrictive to fermentation bacteria during ensiling. For example tannins which occur in some legumes play a major role in inhibiting proteolysis in legume silage (Albrecht & Muck, 1991).

2.6 Animal response to silage

2.6.1 Palatability

Palatability in animals is essential as it affects their production. Buchanan-Smith (1990) defined palatability as a food property, which influences its smell and taste as sensed by animals with particular experiences under specified conditions. Impaired palatability affects the intake problem (Dulphy, Remond, & Theriez, 1980). Although palatability is generally described as a plant characteristic, it needs to be expressed in terms of animal cues that might be different between animal species and their physiological state (Horadagoda, Fulkerson, Nandra, & Barchia, 2009). It is evident that sheep and cattle sometimes have different sensitivity degrees to palatability factors when they are offered a choice of feeds (Marten, 1978).

It is still questionable whether several amino acids, amines or ammonia induces taste or whether odour and taste rank as important elements determining the palatability of these chemicals (Buchanan-Smith, 1990). However, Arnold, & Morley (1981) opined that organic acids, sugars, tannins and alkaloids contribute to palatability. Preconditioning the animals to initially unpalatable species could be performed to improve preferences of grazing sheep. Furthermore, forage plant preferences by ruminants may be due to the sense of sight, touch, taste, smell, and instinct.

(Smit, Tamminga, & Elgersma, 2006) found that WSC had a positive correlation with palatability. (Nombekela, Murphy, Gonyou, & Marden, 1994)stated that WSC is the sweet portion of a plant that increased palatability. The plants with bitter portions, such as plants containing nitrates will be avoided by grazing animals (Provenza *et al*. 2007). Furthermore, plants with high fibre and lignification reduced palatability (Baumont, Prache, Meuret, & Morand-Fehr, 2000). However, other

aspects of the plant should be considered such as alkaloids in fescue, sesquiterpene lactones in chicory, and volatile compounds in herbs that might reduce palatability (Horadagoda *et al*. 2009).

Silage quality affects palatability, feed intake and milk production when supplemented with fresh forage or offered as a sole diet (Castle, Retter, & Watson, 1980). Diet preference is essential to the growth of livestock. High palatability of diets results in high feed intake, which, in turn, affects livestock growth. Furthermore, Lombardi, Vasseur, Berthiaume, DeVries, & Bergeron (2015) stated that forage preference was not affected by harvest time of day but maybe improved by the ensiling process. However, Smit *et al*. (2006) reported that there was a strong correlation between WSC and palatibility whereas the NSC of the plant increased palatability, and NSC is higher during the day (Morin *et al*. 2011).High total nitrogen also results in a high preference silage (Lombardi *et al*. 2015; Tolkamp et al., 1998). Moreover, Van Dorland, Kreuzer, Leuenberger, & Wettstein, (2008) stated that with ensiled forage, the method of feeding had no effect on the number of meals per day.

2.7 Ensiling alternative pasture species

Although ryegrass is very productive in fertile soils with adequate moisture and is less resistant to drought than other pasture species (Ivins, 1952; Mook, Haeck, Van der Toorn, & Van Tienderen, 1989), ryegrass also hosts the fungal endophyte (*Accremonium spp*) which confers protection from insect pests and some drought resistance though the native strain causes staggers in sheep and that can cause losses in animal production (Siegel *et al*. 1985). For solving the challenges of ryegrass pastures, a mixed pasture can be an alternative for ryegrass/clover alone. Woodward *et al*. (2013) stated that mixed pastures, such as perennial ryegrass, white clover, prairie grass, lucerne, chicory, and plantain, have similar annual DM production as conventional ryegrass clover pasture. In fact, mixed pastures can produce higher DM than standard pasture in summer (December, January, February) when deep rooted species such as chicory and plantain are included (Nobilly, 2012). *Plantago lanceolata* L,commonly known as plantain or ribgrass, is a herb that has been bred for improved acceptability by livestock and increased seasonal and annual herbage yields in temperate environments (Stewart, 1996).

In addition to improved summer growth, plantain (*Plantago lanceolata L*) has recently gained increased attention as a forage that may mitigate against high N losses from ruminant excreta in pastoral grazing systems (Woodward *et al*. 2013). Recent research has shown that multi-species - also known as diverse pastures containing additional herbs, such as plantain, chicory and legumes - such as red clover and lucerne - in a pasture mixture with perennial grass and white clover, reduced urinary N concentrations (Edwards *et al*. 2015). Furthermore, Totty *et al*. 2013 reported that there

was lower urine N concentration and excretion in diverse pastures containing plantain compared to a simple perennial ryegrass-white clover mixture. The reasons for the reduction in urinary N concentration and excretion are believed to be associated with high moisture content, though secondary metabolites may also contribute (Bryant *et al*. 2019).

Increasingly farmers are expected to alter their farm systems and adopt new technologies that address the environmental, social, and economic pressures. The evidence which highlights the opportunity to use alternative forages such as plantain as a mitigation tool for nitrogen losses offers some relief to farmers seeking options. However, the adoption of an alternative forage also raises a lot of questions around integration into farm systems. Compared with the traditional pasture species, perennial ryegrass (*Lolium perenne*), plantain differs in every manner in its physical morphology, organic matter content and composition and the presence of secondary metabolites. Ultimately, this results in the variable response of plantain under similar management to ryegrass, and improved understanding will enable improved efficiency in the utilisation of these plants.

Table 2.5. Effect of age and irrigation on the nutritive characteristics of plantain and chicory

Plant	CP	NDF	Source
		Chicory	
-Leaf : - younger	24.3	21.7	
- older	19.1	26.3	(J.M. Lee, Hemmingson,
-Stem : - immature	15.4	28.9	Minnee, & Clark, 2015)
- mature	8.3	48.8	
- with irrigation	24.0	35.3	(Minneé, Clark, & Clark, 2013)
- without irrigation	23.6	34.9	
		Plantain	
-Leaf : - younger	22.1	22.1	
-older	18.6	28.9	(J.M. Lee et al., 2015)
-Stem : -immature	16.1	37.8	
-mature	11.8	47.7	
- with irrigation	22.3	36.2	(Minneé et al., 2013)
- without irrigation	21.2	36.8	

As this literature review has shown, the nutritive composition of the plant such as WSC is important in providing the right conditions for ensiling. There are very few documented studies investigating the ensilability of plantain. To date previous research has found that plantain is difficult to ensile due to its high pH of >5, little lactic acid or acetic acid production. Duru, Sarihan, & Bulduk (2018) who studied plantain silage on a laboratory scale reported that plantain can be ensiled well without additives in terms of chemical content (CP, OM, NDF, ADF). They further stated, they achieved good quality silage with pH of 3.95 using naturalised plantain ensiled in plastic jars, though those authors provided little information about the plantain itself or management. Furthermore, Raeside, Nie, Lamb, Byron, & Behrendt (2012) stated that the timing of herbage and grass silage cutting is crucial and it is likely to improve nutritive value of its silage and this can be achieved by harvesting at the early stage of maturity. For adoption of plantain into pastoral farm systems, more information on how to manage it is required, not simply in terms of grazing but also conserving.

2.8 Conclusion

Review of the literature has shown that there are a number of biological processes which need to occur in order for forages to ensile effectively. Previous research has also identified that there are a number of plant management and environment factors having a large influence on both the fermentation of the silage and subsequent nutritive value. Knowledge gaps are evident in the ability to ensile novel forages such as plantain, which evidently have plant chemistry which differ from conventional grasses and legumes. The purpose of the following experiments are to address those questions around ensilability of plantain and the extent to which some management and environmental factors alter silage quality.

Chapter 3

Effect of maturity and storage duration of plantain (*Plantago lanceolata*) silage harvested in late spring

3.1 Introduction

There is a growing awareness of the negative environmental effects of dairy farming and pressure on farmers to utilise pastures that benefit the environment by reducing nitrogen losses from animals. Increasingly, research is demonstrating that forage plantain may be able to meet this requirement as it is able to contribute to lowering urinary N concentrations on pasture (Box, Edward, & Bryant, 2016; Totty *et al*. 2013; Woodward *et al*. 2013). However, adoption by farmers may be limited by a lack of knowledge of how plantain can be integrated into the farm system. For example, the production of plantain has been observed to be higher than that of perennial ryegrass in summer and autumn (Minneé *et al*. 2013) so management of the surplus plantain may pose a problem. Typically in late spring or early summer, high pasture growth produces a surplus of feed that can be conserved and used as supplementary feed during a time of scarcity (Howse *et al*. 1996). According to Stewart (1996) plantain can be conserved as silage, although a review of the literature (Chapter 2) showed there is little research to support this statement, nor on factors which can be used to manipulate the silage quality. Good quality silage will be only slightly lower in feed value than the original forage.

Basically, there are two aspects that influence the quality of silage; namely, controllable (such as forage species, stage of maturity, type of storage) and uncontrollable factors, such as the season (Mahanna & Chase, 2003), as forage yield, digestibility , CP concentration, mineral composition and functional compounds are affected by climate, directly or indirectly (Bernardes *et al*. 2018). Furthermore, the season determines the non-structural carbohydrates, which are part of the non-fibre carbohydrate where the non-structural carbohydrate (NSC) is the main source of a fermentable substrate during ensiling. If spring and summer temperatures are warm, the increase in day length and thermal time prompts reproductive development in most pasture species. The accumulation of seed heads signifies the presence of stalks, which are not only difficult for livestock to digest but also air spaces are created during ensiling that may promote the growth of unwanted bacteria and fungi in silages.

The stage of maturity, which is a controllable factor, plays a vital role in the quality of silage. The maturation of the plant tissue alters the availability of fermentable sugars, which are important for silage microorganisms as well as the nutritive value for animals (Mahanna & Chase, 2003). Research investigating the effects of the increasing maturity of wheat silage showed a large negative effect on its fermentation quality and nutrition (Xie, Zhang, Chen, Li, & Zhang, 2012).

Another controllable factor, which is no less important, is the storage duration of the silage itself. A study conducted by Saricicek, Yildirim, Kocabas, and Ozgumus Demir (2016) showed that the nutrient composition of corn silage reduced with prolonged storage in a mini silo. Dry matter and aNDF digestibility tended to diminish with time in wheat and corn silage but this pattern was not consistent among silage types (Weinberg & Chen, 2013).

Unfortunately, there has been no research comparing regrowth stage and storage duration on the quality and nutritive composition of plantain silage. The objectives of this study were to investigate the effect of regrowth stage on the fermentation characteristics and nutritive value of plantain (*Plantago lanceolata*) silage in late spring.

3.2 Materials and Methods

3.2.1 Site and experimental design

This study was conducted at the Lincoln University Research Dairy Farm (LURDF) Canterbury, New Zealand (43°38S', 172°27E') in late spring 2016 (14 November - 28 December 2016). The soil type of the experimental site was Paparua silt loam. The soil chemistry status, as determined from soil sampling from the area to 7.5 cm on 04 November 2016, is presented in Table 3.1.The experimental design was a replicated 3 x 4 factorial completely randomised design with three levels of leaf appearance and four levels of storage duration and five silo replicates. The maturity stages were 4-leaf appearance (4L), 5-leaf appearance (5L) and 6-leaf appearance (6L) and the storage durations were 90, 120, 150 and 180 days. Replicates were prepared in mini silos giving a total of 60 mini silo's.

The plantain (cv Tonic) was established in December 2014 following spraying with roundup and cultivation of a five-year old perennial ryegrass pasture. The area was fully irrigated using a centre pivot system. The area had been rotationally grazed by dairy cows to manage herbage mass.

Table 3.1. Soil fertility status of plantain and ryegrass areas in 2016 (LURDF, 2017)

Parameter	Level found	Recommended range
pH	6.2	5.8-6.2
Olsen phosphorus (mg/L)	22	20 - 30
Potassium (me/100 g)	0.89	0.4 - 0.6
Calcium (me/100 g)	8.6	4.0 – 10.00
Magnesium (me/100 g)	1.11	1.00 – 1.6
Sodium (me/100 g)	0.22	0.2 - 0.5
CEC (me/100 g)	15	12 – 25
Total base saturation (%)	71	50 – 85
Volume weight (g/mL)	0.94	0.6 – 1.00
Sulphate sulphur (mg/kg)	10	10 - 12

Silage harvesting

On 14 November 2016 a 6000 m^2 area of plantain pasture was sub-divided into three areas which were randomly designated a leaf stage treatment. The existing plantain sward was mown to 6 cm using a UFO mower and tractor and urea fertiliser was applied at 25 kg N/ha. The area was left to regrow until each treatment maturity stage was reached. The sward reached leaf stage targets of 4L, 5L and 6L on 14, 21 and 28 December, respectively. Before harvest, the leaf stage was confirmed by counting the number of fully developed leaves per shoot on 20 random plants.

At each harvest date, one third of the area was mown to a 6 cm height in the afternoon (between 13.00 and 14.00 h) to maximise sugars. The herbage was then wilted for 24 – 48 hours until the DM% was deemed suitable for ensiling. The suitability for this was based on the "squeeze test", as described by Moran (2005), whereby a sample of herbage was cut into 1-2 cm pieces and squeezed tightly in the hand for 30 to 60 seconds. If the hand became moist but not wet, the DM was assumed to have approximately 30% DM content. Sub-samples of fresh herbage were taken for each treatment immediately after mowing (DM determination) and, again from the wilted forage, immediately before ensiling. The dry matter concentration was confirmed by oven drying a sub-sample of 50 g fresh weight (FW) for 48 hours at 60°C. Approximately 200 g of fresh and wilted herbage was stored in a freezer (-20°C) for later freeze drying and further herbage analysis. Three samples of each fresh plantain and wilted plantain were taken then composited for further analysis.

The DM yield of plantain was measured by harvesting the plant material in three random quadrats (0.2 m^2) for each maturity stage. Weeds and soil were removed from harvested material before the samples were oven dried at 60°C for 48 hours.

Similarly, any weed material was manually removed prior to ensiling. To create the mini silos (five per treatment) approximately 500 g of wilted plantain (leaf and stem) was pressed firmly into a 35 micron polyethylene plastic bag (23 x 38 cm) and the bag was manually pressed and sealed to exclude any air.Plantain herbage was compacted as densely as possible and the plastic bag folded three times before sealing with adhesive tape (Ashbell *et al*. 2001). The plastic bags were twisted again and then retaped (Plate 3.1.a). The mini silo was inserted into a second plastic bag. which also had the air removed, twisted and sealed with adhesive tape. All bags were then stored in black plastic drums in a shed for 90, 120, 150 or180 days.

3.2.2 Herbage analysis

At each treatment storage date, five replicate silos from each leaf stage treatment were removed and the following measurements were carried out for each treatment at each storage duration date. Visual assessment of the silage was conducted by identifying the presence of mould and scoring the percentage of mould growth on the surface area of the mini silo.

The pH and buffering capacity (BC) were determined following the procedure described by Playne and McDonald (1966). Briefly, the pH of silage of was measured by macerating 20 g chopped fresh silage with 250 ml distilled water. After 30 minutes the pH meter (Thermo scientific, USA) was inserted and the pH recorded. The macerated silage sample for pH was then titrated first to pH 3 with 0.1 N hydrochloric acid in order to release the bicarbonate as carbon dioxide and then titrated to pH 6 with 0.1 N sodium hydroxide. The buffering capacity was expressed as meq. of alkali required to change the pH from 4 to 6 per 100 g of dry matter (meq./100 g DM).

For ammonia and volatile fatty acid (VFA), 20 grams of fresh silage were macerated with 100 mL of distilled water and then left to stand for 30 minutes. Two ml of solution from the macerator then had NH3-N, total VFA and lactic acid measured using a Randox kit (Rx Daytona, UK).

The dry matter percentage was determined by weighing approximately 50 g FW sample and oven drying at 60°C for 48 hours. Nutritive characteristics of fresh plantain and plantain silage were determined by wet chemistry using freeze dried samples that were milled through a 1 mm screen

centrifugal rotor mill (Retsch-ZM, Haan, Germany). The botanical composition of the silage was then measured by sorting 25 g into stems and leaves then oven drying for 48 hours at 60°C. The proportion of the leaves and stem were calculated on a DM basis

Acid detergent fibre (ADF) was determined gravimetrically by the addition of 1 g of sample with 50 ml of detergent solution (cetyltrimethylammonium bromide and sulphuric acid), then heated for 1 hour. The detergent was then filtered to waste using hot water before rinsing with acetone. Afterwards, it was dried at 100°C overnight and ashed at 500°C for two hours. The difference in weight between the sample dry weight and the sample ash weight over the sample weight times DM (residue dry matter) was recorded as ADF% (AOAC, 1990).

Neutral detergent fibre (NDF) was determined by extracting 1 g of sample with sodium lauryl phosphate, ammonium pentaborate and EDTA. The NDF% was calculated by subtracting the dry weight with the ash weight over sample weight times rDM (Van Soest, Robertson, & Lewis, 1991).

Crude protein (CP) was based on total N concentration that was measured using an elementor analyser Vario Max CN with catalytic tube combustion under an oxygen supply and high temperature. Crude protein as is was calculated by multiplying total N x rDM x 6.25.

Water soluble carbohydrate (WSC) was analysed for fresh and wilted plantain using the method described by (Pollock & Jones, 1979) where the sample was extracted in ethanol and water for low molecular weight (LMW) and high molecular weight (HMW) using respective sucrose and inulin standards following anthrone reaction.

Secondary metabolites (aucubin, catalpol and acteoside) were analysed for selected treatments using high performance liquid chromatography (HPLC) by the Lincoln University Analytical Laboratory following the procedure of Tamura & Nishibe (2002). In brief, secondary metabolites were extracted from 3 replicates of plantain 4L and 6L silage stored for 90 and 180 days by adding 1.5ml of 100% methanol. Aucubin and catalpol were determined by adding 0.5 ml of the extracted mixture of samples to 0.5 ml of water, mixing well and filtering through a 0.45 µm nylon membrane syringe filter into 2 ml HPLC vials and stored at -20°C before HPLC analysis. Acteoside was measured by filtering the extracted mixture of samples with a 0.45 µm nylon membrane syringe filter without dilution into a HPLC vial, and then stored in -20°C until HPLC analysis.

Dry matter digestibility (DMD) was based on the procedure by Clarke, Flinn, and McGowan (1982). The sample was digested for 48 hours at 50°C with acidified pepsin, incubated at 50°C and then digested for 48 hours at 40°C with a buffered cellulase solution. The disappearance of dry matter or organic matter was determined as *in vitro* DM digestibility.

In vitro DMD (%) = 100 * [(rDM/100)*sW - rW] / [(rDM/100)*sW]
Where:
rDM = % Residual dry matter of sample (determined independently)
sW = sample weight
rW = residue weight

In vitro OMD (%) = 100 * [(rDM/100)*(OM/100)*sW - orW] / [(rDM/100)*(OM/100)*sW]
Where:
OM = % Organic matter of sample (determined independently)
rDM = % Residual dry matter of sample (determined independently)
sW = sample weight
orW = organic residue weight
In vitro DOMD (%) = OMD * (OM/100)
ME (MJ/kg DM) = DOMD * 0.16

3.2.3 Meteorological data

Meteorological data was taken from the National Climate Database (http://cliflo.niwa.co.nz). The growing degree day (GDD) was calculated using mean temperature and thermal requirements of plantain where:

$$GDD = \frac{\text{Temperature maximum + temperature minimum}}{2} - \text{base temperature}$$

The base temperature used for plantain was 5°C based on Powell, Kemp, Jaya, & Osborne (2007).

3.2.4 Statistical analysis

Data collected for fresh plantain was analysed using a one-way ANOVA, with variables of fresh plantain (production, leaves, seed heads and shoots) as fixed terms and the replicates as random terms. This analysis used the statistical package GenStat version 18 (VSN International Ltd, 2015). All data collected from the plantain silages were analysed using two-way factorial ANOVA (3 regrowth stages x 4 storage durations) using the statistical package GenStat, version 18 (VSN

International Ltd, 2015). The model used to analyse the data was stage maturity x storage duration as the fixed term and replications as block or random term.

3.3 Results

3.3.1 Climate conditions

Air temperatures during harvesting at the 4-leaf and 6-leaf emergence were very similar and 5 degrees lower than temperatures at time of harvesting 5L (Table 3.2.). Nonetheless, air temperature during wilting was similar among the treatments at above 20°C.

Table 3.2. Air temperature during harvest and when wilting the herbage

Plantain	Harvest date	Air temperature during harvesting (°C)	Air temperature during wilting (°C)	Ensiling date
4L	14 December 2016	17.4	20.7	15 December 2016
5L	21 December 2016	23.1	20.4	22 December 2016
6L	28 December 2016	17.4	21.1	30 December 2016

*Where 4L = 4-leaf , 5L= 5-leaf appearance, 6L=six-leaf appearance

The average temperatures in this study were 19.6°C and 9.3°C for the maximum and minimum temperatures, respectively (Figure 3.1). The growing degree day (GDD) for plantain 4L was 373.4, for plantain 5L was 471.3 and plantain 6L was 572.9.

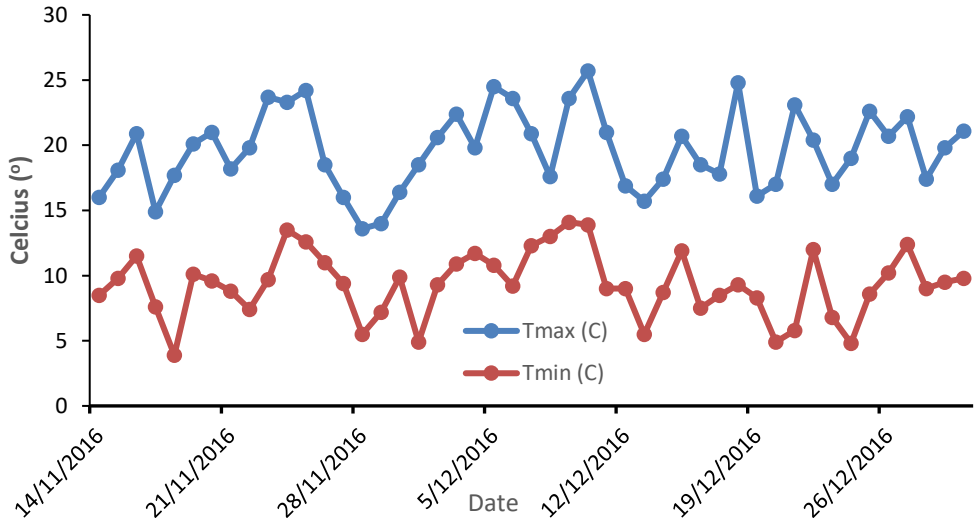

Figure 3.1. Temperature maximum and minimum from 14 November – 30 December 2016 for plantain. Data was taken from the National Climate Database (Cliflo).

3.3.2 Plantain characteristics

Large variance between quadrat cuts prevented detection of differences in yield among the regrowth stages (Table 3.3), though numerically yield at 6L was roughly 1000 kg DM/ha greater than at 4L or 5L. The distribution of yield between leaves and shoots is characterised in Table 3.3. Because the plantain pasture was two years old, plantain plants consisted of multiple shoots with variable age structure which influenced the size of the shoot and number of flowering stems. Nonetheless, shoot number and seed head production increased with the increasing regrowth interval. At the time of harvest, in December, there were many stems and seed heads in plantain plants, averaging 50-55% for the 4L and 5L crops but for 6L it was more than 70% (Table 3.3).

3.3.3 Nutritive value of fresh plantain

Nutritive content of the plantain (fresh or wilted) were relatively poor with digestibility of the fresh herbage less than 70% and ME concentrations at 10 MJ of ME per kg of DM or lower. Across the different treatment growth stages the nutritive parameters (i.e. digestibility and ME) for plantain differed by less than 5%. Crude protein, and OM that decreased with increasing regrowth stage (Table 3.4). The DM content increased with the increasing regrowth interval. As expected, wilting increased the DM content of plantain forage, so all plantain was between 30 and 40% of DM prior to ensiling. Crude protein and WSC, which form the major substrates for LAB were both relatively low. The secondary metabolites of fresh plantain show that at the early regrowth stage, the percentage of secondary compounds were higher than those of late harvested.

Table 3.3. Plantain characteristics in late spring

Treatment	Production (kg DM/Ha)	Number of leaves/plant (leaves)	Number of shoots/plant (shoot)	Number of seedheads/plant (seedhead)
Plantain 4L	1476	3.98[c]	10.9 [a]	19.1 [a]
Plantain 5L	1546	5.00 [b]	12.6 [a]	25.2 [ab]
Plantain 6L	2435	6.03[a]	15.5 [b]	30.1 [b]
SEM	428.4	0.089	0.68	2.16
P value	0.153	<.001	<0.001	0.003

*Where 4L = four leaves appearance, 5L = five leaves appearance and 6L = six leaves appearance
Different notations in the same column show significant results (P<0.001), SEM = standard error of means

Table 3.4. Nutritive characteristics (% of DM) of fresh and wilted plantain four leaves appearance (4L), five leaves appearance (5L) and six leaves appearance (6L) prior to ensiling.

Variable	Plantain 4L		Plantain 5L		Plantain 6L	
	Fresh	Wilted	Fresh	Wilted	Fresh	Wilted
Dry matter (%)	18.9	38.1	22.2	30.4	22.2	35.8
Organic matter (%)	81.1	90.7	90.4	90.1	90.1	90.3
Neutral detergent fibre (%)	34.2	44.7	39.5	43.2	43.6	45.3
Acid detergent fibre (%)	25.1	32.1	27.9	31.6	31.1	32.3
Crude protein (%)	12.2	11.3	11.7	11.1	10.4	9.6
Water soluble carbohydrate (%)	3.01	4.12	3.63	3.5	4.66	4.03
DM digestibility (%)	68.5	66.7	68.1	66.7	65.6	64.5
OM digestibility (%)	69.4	67.2	69.5	68.5	67.5	66.2
Digestible OM in the DM (%)	56.3	60.9	62.8	61.7	60.8	59.8
Metabolisable Energy (MJ ME/kg DM)	9	9.74	10	9.87	9.73	9.57
Aucubin (mg/g)	0.34	-	-	-	0.21	-
Catalpol (mg/g)	1.58	-	-	-	1.40	-
Acteoside (mg/g)	18.16	-	-	-	16.49	-

Where OM is organic matter, DM is dry matter, MJ ME/kg DM= Megajoule metabolisable energy/kilogram dry matter, mg/g = milligram/gram

3.3.4 Fermentation characteristics of plantain silage

Upon opening the mini-silo's at each storage date assessment was made of each bag. Visibly the silage did not look attractive as the ensiled forage was very dark brown in colour and many of the silo's had mould growing on the outer surface (Plate 3.1). However, the mould was mainly restricted to air pockets on the outside of the silage and the silage had a sweet smell. The mould found in this study was *Byssochlamys nivea* which is responsible for spoilage and degradation of silage.

In general, the pH was high with a mean of 5.16. The pH of the silage was affected by regrowth stage ($P<0.05$) where the highest pH was reached at 6L. However, there were no interactions between treatments, and storage duration had no effect on the pH of silage plantain.

a).

b).

c).

d).

Plate 3.1. Mini silo of plantain (a), the performance of plantain 4L silage (b) plantain 5L silage (c) and plantain 6L silage (d) stored for 180 days

In terms of BC, 4L and 6L had better value (± 162) compared to 5L, which was more than 200. Prolonged storage reduced the BC value and there was an interaction between stage of maturity with storage duration, with the highest result reached at 4L stored for 150 and 180 days. Significant results of total VFA, lactic acid and propionic acid were found at the early regrowth stage. However, total VFA decreased with prolonged of storage duration. There was an interaction between regrowth stage and storage duration for total VFA.

However, the percentages of lactic acid and propionic acid were unaffected by storage duration. There was an interaction between regrowth stage and storage duration in lactic acid percentage. Especially for propionic acid, which was only influenced by regrowth stage. Generally, the butyric acid percentage was below 0.5% and there were no differences among the treatments carried out. The amount of NH_3N was also good at below 1%, and it was unaffected by regrowth stage but was affected by storage duration. There was no interaction between regrowth stage and storage

duration with the NH_3-N value. Although a number of these fermentation characteristics were low, sensory analysis of the silages upon opening confirmed low butyric acid and ammonia levels, with silages conferring a sweet smell.

In general, the pH was high with a mean of 5.16. There were no interactions between growth stage treatments and storage duration for pH of the silage which was predominantly affected by regrowth stage ($P<0.05$) where the highest pH was reached at 6L. However, there were no interactions between treatments, and storage duration had no effect on the pH of silage plantain. The later growth stage resulted in higher pH

An interaction between regrowth and storage for BC showed that in general BC did not change with storage duration with the exception of 4L which was had a reduction in BC between 90 and 120 days storage. In terms of BC, both 4L and 6L had better value (162 meq./100g DM) compared to 5L, which was more than 200 meq./100g DM.

Total VFA generally decreased with prolonged of storage duration though an interaction with regrowth stage showed that at 5L there was no effect of duration on total VFA. The variation in total VFA were largely driven by similar interactions for both acetic and lactic acids. Generally, the butyric acid percentage was below 0.5% and there were no differences among the treatments. The amount of NH_3N was also good at below 1%, and it was unaffected by regrowth stage but was affected by storage duration. There was no interaction between regrowth stage and storage duration for NH_3-N.

Table 3.5. Fermentation characteristics of plantain four leaves appearance (4L), five leaves appearance (5L), six leaves appearance (6L) silage (% DM)

Treatment	Storage (day)	BC (meq./100g DM)	pH	Total VFA (%)	Acetic acid (%)	Butyric acid (%)	Lactic acid (%)	Propionic acid (%)	NH_3-N (%)	Stem proportion (%)	Visual assessment of mould (%)
4L	90	210.3 ^{b}	4.91	1.01 ^{a}	0.43 ^{a}	0.03	0.54 ^{a}	0.00	2.05	52.7	20 ^{ab}
	120	157.7 ^{a}	5.05	0.56 ^{bcd}	0.22 ^{e}	0.01	0.27 ^{bcd}	0.00	1.45	48.1	20 ^{abc}
	150	133.7 ^{a}	4.95	0.80 ^{abcd}	0.39 ^{abc}	0.05	0.29 ^{bcd}	0.01	1.56	51.8	18 ^{a}
	180	146.3 ^{a}	5.13	0.58 ^{bcd}	0.26 ^{cde}	0.01	0.25 ^{bcd}	0.00	1.61	50.4	20 ^{abc}
5L	90	218.1 ^{b}	4.99	1.00 ^{a}	0.41 ^{ab}	0.03	0.46 ^{ab}	0.02	1.89	53.0	24 ^{abcd}
	120	223.4 ^{b}	4.95	0.94 ^{ab}	0.36 ^{abcd}	0.02	0.45 ^{abc}	0.02	1.85	49.3	25 ^{abcd}
	150	217.5 ^{b}	4.99	0.85 ^{abc}	0.30 ^{bcde}	0.07	0.40 ^{abc}	0.02	2.01	56.4	29 ^{de}
	180	210.3 ^{b}	5.00	1.11 ^{a}	0.86 ^{abcd}	0.05	0.59 ^{a}	0.03	2.38	59.5	34 ^{ef}
6L	90	163.4 ^{a}	5.52	0.57 ^{bcd}	0.24^{de}	0.02	0.17 ^{a}	0.03	2.25	73	28 ^{bde}
	120	164.7 ^{a}	5.34	0.62 ^{bcd}	0.21 ^{e}	0.13	0.17 ^{a}	0.02	1.57	72.5	31 ^{de}
	150	166.2 ^{a}	5.49	0.54 ^{cd}	0.20 ^{e}	0.04	0.24 ^{cd}	0.01	2.02	66.5	39 ^{f}
	180	155.9 ^{a}	5.52	0.45 ^{cd}	0.18 ^{e}	0.28	0.18 ^{d}	0.01	1.75	73.7	47 ^{g}
SEM		11.9	0.66	0.009	0.03	0.03	0.51	0.007	0.06	6.51	1.90
Regrowth		<0.001	<0.001	<0.001	<0.001	0.30	<0.001	0.007	0.009	<0.001	<0.001
Storage		0.04	0.59	0.048	0.001	0.48	0.12	0.81	0.02	0.07	<0.001
Regrowth x Sorage		0.031	0.69	0.005	0.007	0.08	0.003	0.18	0.06	0.77	0.001

*Different notation in the same column differed significally (P<0.05), SEM = standard error of means, BC = buffering capacity, meq = milli equivalent, g = gram, DM = Dry matter VFA = volatile fatty acid, NH_3-N = ammonia nitrogen

3.3.5 Nutritive value of plantain silage

The dry matter percentage of plantain silage was affected only by regrowth stage ($P<0.001$), where 4L had the highest DM percentage followed by 6L then 5L (Table 3.6.) For organic matter, the value was below 90% and increasing regrowth stages resulted in higher organic matter contents. The organic matter was affected by regrowth stage and storage duration. As expected, ADF and NDF increased with the increased regrowth stage and storage duration. All ADFs of plantain silage were below 40%, whereas the NDF were below 52%. There was an interaction between maturity and storage duration for ADF and NDF. Conversely, crude protein was reduced by the delay in harvest time and the crude protein was only influenced by regrowth stage. Although storage duration did not change the value of the silage protein, the average crude protein percentage was quite low (<15%).

The highest value of DM digestibility of plantain silage was lower than 67%. This was at the 4L regrowth stage and stored for 90 days. DM digestibility was reduced by both extension of the regrowth stage and extension storage duration. Because calculation of ME was based on digestibility, the ME value was also low (<10 MJ ME/kg DM) and was affected by treatments in a similar manner as digestibility.

For the secondary metabolites, aucubin and catalpol, all disappeared during ensiling. However, acteoside had a tiny amount remaining at the 4L plantain silage, accounting for 0.05% at 90 days of storage duration and more than four times higher at 180 days. Whereas acteoside in the 6L plantain stored for 90 days was not different with acteoside at 4L stored for 90 days. However, the content of acteoside decreased at 6L stored for 180 days. There also was an interaction between treatment and storage duration where the highest content of acteoside was at plantain 4L stored for 180 days

Table 3.6. Effect of regrowth stage and storage duration to nutritive value of plantain four leaves appearance (4L), five leaves appearance (5L) and six leaves appearance (6L) silage

Treatment	Storage duration (day)	DM (%)	OM (%)	NDF (%)	ADF (%)	Crude protein (%)	DMD (%)	DOMD (%)	ME (MJME/ kgDM)	Aucubin (mg/g)	Catalpol (mg/g)	Acteoside (mg/g)
4L	90	36.3	84.4 ^{b}	40.3	28.5 ^{g}	16.5 ^{f}	66.9 ^{d}	57.0	9.18	0	0	0.53 ^{b}
	120	37.9	81.7 ^{a}	42.1	31.1 ^{f}	13.9 ^{e}	62.2 ^{bc}	53.5	8.56	-	-	-
	150	36.8	85.6 ^{bc}	44.8	32.1 ^{ef}	14.2^{e}	62.7 ^{bc}	55.1	8.81	-	-	-
	180	38.7	85.7 ^{bc}	45.6	33.0 ^{def}	13.4 ^{de}	62.2 ^{bc}	55.0	8.80	0	0	2.27 ^{a}
5L	90	27.7	88.6 ^{d}	45.9	34.2 ^{cde}	13.1 ^{bcde}	62.7 ^{bc}	54.5	8.72	-	-	-
	120	28.0	88.6 ^{d}	46.2	36.1 ^{bc}	14.1 ^{e}	64.2 ^{cd}	56.9	9.11	-	-	-
	150	28.2	87.9 ^{cd}	46.8	34.6 ^{cd}	13.3 ^{cde}	61.2 ^{bc}	53.8	8.60	-	-	-
	180	28.7	88.0 ^{cd}	48.3	36.0 ^{bc}	13.0 ^{abcd}	60.2 ^{b}	53.0	8.48	-	-	-
6L	90	33.4	88.9 ^{a}	51.5	39.3 ^{a}	11.4 ^{a}	55.1 ^{a}	48.4	7.74	0	0	0.53 ^{a}
	120	30.8	87.6 ^{cd}	50.9	38.2 ^{ab}	11.5 ^{ab}	55.7 ^{a}	47.2	7.55	-	-	-
	150	32.9	88.1 ^{cd}	52.0	38.0 ^{ab}	11.6 ^{abc}	53.0 ^{a}	45.4	7.27	-	-	-
	180	32.5	88.5 ^{d}	52.8	39.6 ^{a}	11.8 ^{abcd}	54.4 ^{a}	47.6	7.6	0	0	0.39 ^{b}
SEM		1.36	0.61	0.91	1.08	0.77	0.95	0.72	0.23	-	-	0.07
Regrowth		<0.001	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	<0.001
Storage		0.80	<0.024	<0.001	<0.009	0.221	0.002	0.145	0.145	-	-	0.002
Regrowth X Storage		<0.78	<0.001	0.306	0.033	0.021	0.036	0.065	<0.065	-	-	<0.001

*Different notation in the same column differed significantly (P<0.05), SEM=standard error of means, DM = dry matter, OM=Organic matter, NDF=neutral detergent fibre, ADF=acid detergent fibre, DMD=dry matter digestibility, DOMD=Digestible organic matter in dry matter, ME=metabolisable energy, MJ=mega joule, mg/g=milligram/gram, SEM=standard error of means, P = probability

3.4 Discussion

The hypotheses for this current study were that farmers could use pre and post harvest management to manipulate the quality of plantain silage. Many farmers often forego quality in favour of yield to minimise costs of ensiling per kg DM. The results of this study showed that the fermentation characteristics of spring-harvested plantain appear dubious, irrespective of stage of maturity. However, there were aspects of both pre and post harvest management which influenced the fermentation and nutritive characteristics of the plantain silage.

3.4.1 Fermentation characteristics of plantain silage

The basic principle of making silage is to stimulate and sustain intensive and lasting lactic acid fermentation to depress plant enzyme activities and unwanted microorganisms by creating low pH under anaerobic conditions (Bolsen, Ashbell, & Weinberg, 1996). Adequate preservation of silage is critical to reduce spoilage by yeasts, moulds and harmful bacteria and to prevent loss of DM. To achieve quality silage ideally the pH should reduce to 4.7 (Muck & Shinners, 2001). The pH of silage and lactic acid concentration from LAB are closely linked. However, there are a number of factors which will influence the activity of LAB and subsequently lactic acid production and pH. These include substrate supply for example, fermentable carbohydrates and protein, buffering capacity and antimicrobial effects i.e. secondary compounds.

The target for low pH requires rapid growth of LAB and the subsequent production of lactic acid. In this study, the high pH corresponded to lactic acid concentrations of lower than 1% of DM. The growth of LAB may have been inhibited either by the lack of supply of readily fermentable sugars, as the WSC was less than 5%. The other reason limiting LAB activity is likely due to the substrate supply being sorbitol sugar (Jiang *et al*. 2019) which is not a common sugar source for silage bacteria. Similarly, protein was also low and potentially unavailable as a proportion of the protein would have been bound in stem material.

A considerable challenge with the mini-silos was the exclusion of oxygen. Using the mini-silo method in this study, the observed mould percentage was high and increased with increased regrowth stage, indicating presence of oxygen. The high proportion of stem was a major contributor to mould growth as the stalks were difficult to compress and remove air, at the late regrowth stage the stems were increasingly lignified so it was more difficult to exclude air from the silo. Retrospectively, it is

likely that the plastic bags used to ferment the silage may not have prevented the ingress of oxygen. Borreani *et al.* (2007), showed that even at plastic wrapping greater than 100 μm, oxygen ingress occurred. The two bags used in the mini-silo experiment was at best 70 μm thickness, and likely allowed ingress of oxygen, particularly at longer storage duration. The silage in this study had a high pH and BC which is likely to affect the aerobic stability of silage when exposed to air. This can be occurred by affecting pH rise following the initiation of aerobic microbial activity on the exposure of silage to air (Wilkinson & Davies, 2012). A common indication of silage which has spoiled is detected through the odour, particularly if butyric acid is high, giving an unpleasant smell. Ideally, where the percentage of butyric acid for high quality of silage fermentation should be between <0.5 – 1% (Kung & Shaver, 2001). In this study the silage had sweet aroma, coupled with a low butyric acid content (below 0.5%), it supports the opinion that plantain is able to be safely ensiled at a relatively high pH.

There appeared to be an association between pH and CP. The high initial pH may have inactivated plant proteases, reducing the extent of protein degradation and maintaining a higher pH. Restriction of proteolysis could be caused by anti-microbial compounds, such as aucubin or acteoside that may have limited microbial activity and/or the slow degradability of plant proteins (Isselstein & Daniel, 1996 *cited in* Seng *et al*. ,2008). Evidence of slow protein degradation by rumen bacteria has also been demonstrated in *in vitro* fermentation studies (Navarrete, Kemp, Paine, & Back, 2016). The limitation might have occurred at the beginning of the fermentation phase since secondary compounds essentially disappeared during storage duration.

Longer storage periods did not cause a decrease in pH and or butyric acid, but NH_3-N slightly decreased. This meant that plantain silage can be stored as long as 180 days without significant damage in fermentation characteristics. This result was consistent with Stewart (1996), who stated that plantain silage still had an agreeable aroma even after 180 days.

Although acetic acid is important for aerobic deterioration, in this study the acetic acid percentage did not change with a longer storage time (<0.4%). The biggest treatment effect on acetic acid occurred due to the regrowth stage where 6L had higher acetic acid than 4L and 5L. This can be explained by the higher levels of oxygen entering the mini silo in the delayed harvest. Moreover, acetic acid was converted from lactic acid by carbohydrates in microorganisms and by a prolonged storage time (Lindgren & Dobrogosz, 1990).

In relation to propionic acid, the higher value was at 5L although it was a tiny amount (below 0.02%) and nothing changed with the length of storage. Weinberg & Muck (1996) speculated that propionic bacteria are only effective in improving the aerobic stability in slowly acidifying silages, because the available strains were sensitive to low pH values and did not produce propionic acid below pH 4.8 (Pahlow & Honig, 1994).

Finally, the high pH of these silages could be explained by activity of LAB which may have been restricted by antimicrobial compounds (Isselstein, 1993 *cited in* Stewart, 1996). Natural plantain contains 1.0-2.7% of aucubin and 1.5 -4.1 5% of acteoside (Tamura & Nishibe, 2002). In Tonic plantain Box & Judson (2018) observed aucubin, acteoside and catalpol levels of <0.5%, 0.1-3.6% and <0.01% respectively. The concentration of aucubin and acteoside in the fresh forage in the current study, were in a similar range to those reported previously, and accounted for less than respective 0.1% and 1.8%. All secondary metabolites (catalpol, aucubin and acteoside) experienced a marked decrease following harvest and ensiling. While Tamura & Nishibe (2002) showed a decline in secondary compounds during wilting, they also found that the decline did not lead to complete losses reaching a stable level within 2-3 hours after cutting. It seems evident that the decrease in secondary compounds observed in this study continued during the ensiling process as ensiling produced heat (Pahlow *et al*. 2003) and that may have destroyed the secondary metabolites. Alternatively, the decrease in acteoside might because acteoside contained sugars (glucose and rhamnose) that were used as energy sources by microorganisms to produce VFA in silage (Rønsted, Göbel, Franzyk, Jensen, & Olsen, 2000).

3.4.2 Nutritive value of plantain silage

Nutritive value of feeds is a biological measure of a nutrient to support metabolic processes (Blaxter, 1956) and often digestibility or metabolisable energy content of feeds is used to define nutritive value (CSIRO, 2007) . The plantain silage in the current study had poor NV as reflected by the low digestibility (53 to 67% DMD) and ME (7.6 to 9.2 MJ ME/kg DM). High quality silage is important because it is often used to support 30-50% of an animals energy requirements when fed as a supplement (Castle *et al*. 1980). Feeds that have low digestibility i.e. <70% will be less able to support both maintenance and production of the livestock they feed (Waghorn, Burke, & Kolver, 2007). Poor quality silage can be attributed to the original harvested forage and how it was managed, or it could be due to nutritive losses during the fermentation process.

In this study, early harvest (4L) of plantain in spring resulted in better quality silage than later harvested plantain (5L and 6L). Consequently pre harvest management had a large effect on quality. The DMD of this plantain silage was around 65% while the expected desired level should be > 70% (Howse *et al*. 1996). At 65% DMD, our results showed higher digestibility than those reported previously for plantain. Raeside *et al*. (2012) found DMD of 56% using spring harvested plantain. Those authors attributed the poor digestibility of their silage to the plant maturity of the fresh forage which they described as being stalky and containing dead material. The results in the present study confirm those of Raeside *et al*. (2012) showing a rapid decline in digestibility of both fresh forage and silage as maturity advanced from 4L to 6L. The low digestibility of plantain silage in this study was likely explained by the high seed head content and of NDF, which exceeded 40% of the DM. In the current study, where plantain was harvested in December, seed heads were prevalent, particularly at the later leaf stages and this it has previously been shown to lead to high NDF and poor DMD (Lee, Minnee, & Clark, 2015). The negative effect of flowering on silage quality was also noted by Howse *et al*. (1996) in their survey of ryegrass silages. They stated that any harvests occurring after November were of poorer quality as seed heads started to develop.

Another aspect of nutritive value is whether feeds can meet the protein requirements of livestock in addition to their energy requirements. The protein content of fresh plantain in this study was low, below 13%, even at the early stage (4L) of maturity. Interestingly the CP% of the silage was slightly higher than the CP% in the fresh forage. The reason for this is likely to be the result of high use of sugar substrates relative to N substrates by silage bacteria. Of the chemical components of the fresh wilted silage, LAB bacteria predominantly use non-structural carbohydrates as their energy source. As a result, this fraction decreases resulting in an increase in protein or fibre fractions, or both. Our value was lower than the plantain CP content reported by Box (2017), using the same experimental pasture two years prior, that plantain had a protein percentage of more than 20%. The plantain used by Box (2017) was a new pasture and did not contain the same amount of stem as that of the present study. The low protein content observed here is the result of high stem relative to leaf and low N fertiliser history.

Finally, nutritive value is also reduced by post harvest management and fermentation factors. Typically DMD also decreases with longer storage durations (Weinberg & Chen, 2013). In this study, the best duration for keeping plantain silage was up to 120 days. In this study, if we calculate the average storage duration of 90 days and 120 days, we will get DMD of plantain silage of 90 days was 63.3% (a), and the DMD of 120 days was 61,2 % (ab). Whereas the DMD of storage duration of 150

days was 58.8% (a) and 180 days was 59.1% (a). It meant that the plantain silage could be stored until 120 days. The digestibility declined with the most significant decrease in of 7% of DMD plantain silage between 150 to 180 days of storage duration. Prior to 120 days, the DMD decreased by about 5% between 90 and 120 days compared to the DMD of wilted plantain. The drop in DMD is largely due to DM losses from digestible material as microbes convert sugars into VFA and CO_2 which cannot later be used by rumen microbes (Buckmaster, Rotz, & Martens, 1989). While storage duration reduced energy availability it did not however affect crude protein content.

3.5 Conclusion

Generally, the fermentation characteristics and nutritive value of plantain harvested for silage in late spring showed poor fermentation and poor nutritive value (based on digestibility and protein content). In spite of fermentation properties being less than recommended levels, the plantain silage smelt sweet and did not seem to be spoiled, indicating safe fermentation. Fermentation characteristics and nutritive value of plantain at the early stage of maturity (Plantain 4L) were better than that of at the late stage of maturity (plantain 6L). Besides, the best storage duration of plantain silage was not more than 120 days. Thus, ensiling plantain at the early stage of maturity is recommended to be used at the commercial scale.

Chapter 4

Effect of maturity and storage duration on the ensiling properties of autumn harvest plantain

4.1 Introduction

In Chapter 3, the effect of plantain stage of maturity and storage duration on spring harvest plantain silage was investigated and found that a large proportion of the dry matter consisted of seed heads, which contributed to poor quality silage (Section 3.3.4). Silages are made any time there is a feed surplus, and while surpluses are common in late spring and early summer, they can also occur in early autumn when rain events follow dry conditions. In autumn, the temperatures are low and thermal time accumulates relatively slowly compared with the conditions experienced in spring. The quality of many plants, including plantain, are often higher during autumn compared to spring or summer, as vegetative growth is predominant (Box *et al*. 2016).

Ensiling a forage will rarely improve the quality of the raw material, so management of forages prior to harvest has important implications on what happens when the forage is ensiled. The climate and soil fertility conditions at the time of harvest can have a dramatic effect on the quality; for example, protein content can be influenced by mineralisation rates and soil N availability (Mahanna & Chase, 2003). Similarly, day length and temperature can affect the accumulation of WSC (Smith *et al*. 1998). In autumn, the temperatures are low and thermal time accumulates relatively slowly compared with the conditions experienced in spring.

Secondary compounds in plantain, such as aucubin and acteoside, have been implicated in environmental benefits, such as reduced urinary N losses (Box, Edwards, & Bryant, 2017; O'Connell, Judson , & Barrell, 2016) and soil nitrification inhibitors (Judson, Fraser, & Peterson, 2019). Recent research has shown seasonal variations in secondary compounds in plantain plants with high concentrations occurring in autumn (Box *et al*. 2016; Navarrete *et al*. 2016). Although autumn is not a typical period for a feed surplus, there may be opportunities to capture quality and environmental benefits from the ensiling plantain at this time.

Therefore, it is proposed that if plantain is to be used as a specialty crop, then ensiling at the appropriate stage of growth during autumn may influence the efficacy of the silage as a feed source. However, there is currently no information about the ensiling properties of *Plantago lanceolata*

during autumn. The objective of this study was to investigate the influence of maturity stage and storage duration on the ensiling properties of plantain during autumn. The hypothesis of this study was that the maturity stage and storage duration influence the fermentative characteristics, nutritive value and digestibility of ensiled plantain.

4.2 Materials and Methods

4.2.1 Site and Experimental Design

This study was conducted at the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand (43°38'S, 172°27'E) in autumn 2017 (19 March – 8 May 2017).The experimental area used was the same as described in Chapter 3. The pasture was managed by rotational grazing using dairy cows. The soil fertility status was described in Table 4.1.

The experimental design was a 3 × 4 factorial completely randomised design with three levels of leaf appearance and four levels of storage duration and five replicates. The maturity stages were four-leaf appearance (4L), five-leaf appearance (5L) and six-leaf appearance (6L), and the storage durations were 90, 120, 150 and 180 days. Replicates were made in mini silos. On 19 March 2016 a 6000 m^2 area was mown to 6 cm and urea fertiliser was applied at 25 kg N/ ha. The area was left to regrow until each treatment maturity's time was reached. The sward reached leaf stage targets 4L, 5L and 6L on 19 April, 26 April and 5 May, respectively. Prior to harvest, the leaf stage was confirmed by counting the number of fully developed leaves/shoot, shoots and seedhead/plant. Twenty samples of plants were chosen randomly for each treatment.

The DM production of plantain was calculated by using a quadrat cut of 0.2 m^2. Samples were taken in triplicate for each treatment. The samples were then oven dried at 60^oC for 48 hours. Pasture mass was estimated using Excel by entering the weight and RPM of the samples, then multiply the g of DM by 50 to achieve kg DM/Ha.

All the harvested plantain was subjected to ensiling, accounting for five replicates for each treatment. With the humid autumn, the fresh plantain was wilted as dry as possible. Any other material, except plantain was removed prior to ensiling. To create the mini silos (five per treatment), 500 g of wilted plantain was subjected to ensiling. The size of the plastic bag used for a mini silo was 23 x 38 cm. The plastic bag was pressed manually to exclude any air, then twisted and sealed using adhesive tape. This mini silo was then inserted into a second plastic bag. In the second bag, the air

was removed, and the bag was twisted and sealed again with adhesive tape (Ashbell *et al*. 2001). All mini silos were then stored in a black plastic drum. The plastic drum was placed in a shed. All mini silos were stored for 90, 120, 150, and 180 days. Mini silos were used as replicates.

Table 4.1. Soil fertility status of plantain and ryegrass areas in 2017 (LURDF, 2017).

Parameter	Level found	Medium range
pH	6.4	5.8 - 6.2
Olsen phosphorus (mg/L)	23	20 – 30
Potassium (me/100 g)	1.08	0.4 - 0.6
Calcium (me/100 g)	8.6	4.0 – 10.00
Magnesium (me/100 g)	1.09	1.00 – 1.6
Sodium (me/100 g)	0.16	0.2 - 0.5
CEC (me/100 g)	14	12 – 25
Total base saturation (%)	76	50 – 85
Volume weight (g/mL)	0.92	0.6 – 1.00
Sulphate sulphur (mg/kg)	3	10 – 12

4.2.2 Herbage analysis

After reaching the determined number of storage days, the silage was removed from the mini silo and measurements were carried out, as described in Chapter 3. For further details, visual assessment was used to estimate the percentage of mould appearing on the surface of silage, while pH and BC were on the fresh silage was measured using the method described by Playne & McDonald (1966).

The percentage DM was measured by oven drying a 50 g sample at 60^{o}C for 48 hours. The botanical composition was determined by separating 25 g of silage into stems and leaves then oven drying for 48 hours at 60°C. The proportions of leaves and stem were calculated on a DM basis. Lactic acid and NH_3-N were measured using a Randox kit (Rx Daytona, UK) and the total VFA of the samples were analysed using gas chromatography (GC-2010, Shimadzu, Japan).

The nutritive characteristics of fresh plantain and plantain silage were determined by wet chemistry using freeze dried samples that had been milled through a 1 mm screen centrifugal rotor mill (Retsch-ZM, Haan, Germany). Acid Detergent Fibre (ADF) was determined gravimetrically following the AOAC (1990) procedure. Neutral Detergent Fibre (NDF) was determined using the method of

Van Soest *et al*. (1991). The water soluble carbohydrate was analysed based on the method described by Pollock & Jones (1979), and for this study, WSC analyses was done for fresh and wilted plantain only. Dry matter digestibility (DMD) was based on the procedure of Clarke *et al*. (1982). Crude protein (CP) was based on the total N concentration that was measured using the elementor Vario Max CN analyser. Crude protein was calculated by multiplying the total N x rDM x 6.25. Where rDM is residue dry matter. For the secondary metabolites, three each of random silo samples from 4L and 6L, after being stored for 90 and 180 days, were subjected to analysis for secondary metabolites (aucubin, catalpol and acteoside). These were analysed by using the HPLC-based method described by Tamura & Nishibe (2002).

4.2.3 Meteorological Data

Meteorological data was taken from the National Climate Database (http://cliflo.niwa.co.nz). The growing degree day (GDD) was calculated using mean temperature and thermal requirements of plantain where:

$$GDD = \frac{\text{Temperature maximum + temperature minimum}}{2} - \text{base temperature}$$

The base temperature used for plantain was 5°C based on Powell *et al*. (2007)

4.2.4 Statistical analysis

Data collected for the fresh and wilted plantain were analysed by one-way ANOVA using GenStat statistical package version 18 (VSN International Ltd, 2015), where the regrowth stage was the fixed term and the mini silo was the random term.

All data collected from the plantain silages were analysed using two-way factorial ANOVA (three regrowth stages x four storage durations) using the GenStat, version 18 (VSN International Ltd, 2015. The stage of maturity and storage duration were fixed terms and replicates as random terms.

4.3 Results

4.3.1 Climate condition

The highest temperature occurred at the end of March 2017 and dropped to a minimum by the end of April 2017 (Figure 4.1). The accumulated growing degree days (GDD) for each stage of maturity were 328; 379 and 426 for 4L, 5L and 6L, respectively.

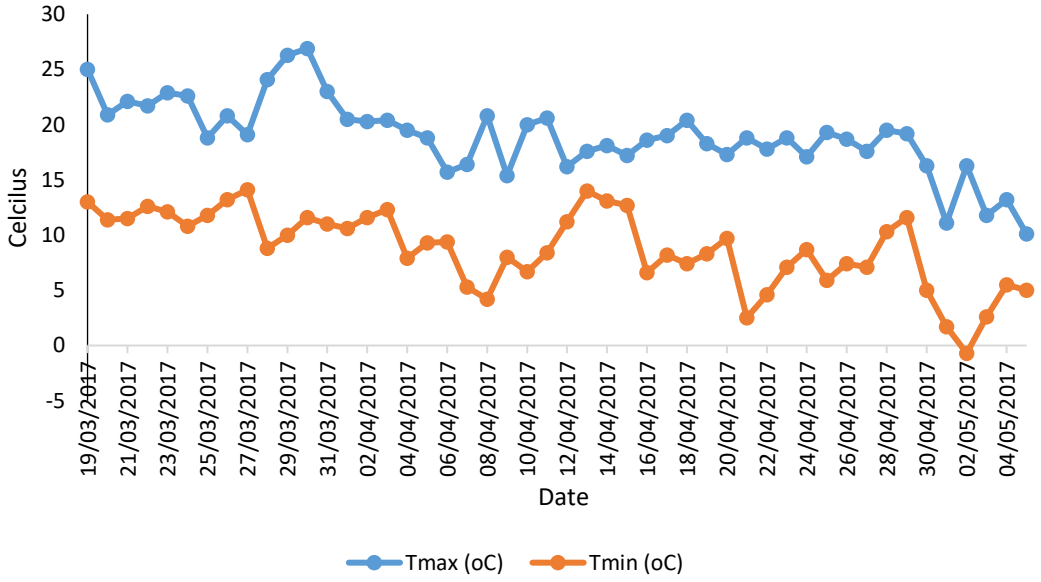

Figure 4.1. Maximum and minimum air temperatures at experimental sites from 19 April – 5 May 2017.

The harvest and ensiling dates for each treatment are provided in Table 4.2. The climate conditions at the time of harvest showed that in May when forage was harvested at the 6L stage, temperatures were approximately five degrees cooler than during the previous harvests at 4L and 5L (Table 4.2).

Table 4.2. Air temperature, harvest date and ensiling date for plantain at four leaves appearance (4L), five leaves appearance (5L) or six leaves appearance (6L).

Treatment	Harvest date	Air temperature during harvest (°C)	Air temperature during wilting (°C)	Ensiling date
4L	19 April 2017	18.3	20.9	20 April 2017
5L	26 April 2017	18.7	22.1	28 April 2017
6L	5 May 2017	10.1	15.8	8 May 2017

For the plantain characteristics of the original pasture (Table 4.3), there were minor differences among the treatments both for production and shoots/plant at this stage there were no seed heads produced even at 6L, except the total leaves per shoot. Dry matter production among the treatments during autumn was very low, at less than 1600 kg/ha, with a range of 975-1690 kg/ha. In spite of differences in growing degree days between 5L and 6L there was no yield difference. Yields were mainly driven by increasing leaf number but at advancing maturity in autumn there was a coinciding drop in shoot number.

Table 4.3. Plantain characteristics for plantain at four leaves (4L) five leaves (5L) or six leaves (6L) appearance.

Treatment	Production (kg DM/Ha)	Number of leaves/plant (leaves)	Number of shoots/plant (shoot)
Plantain 4L	1147	4.08 [c]	7.7
Plantain 5L	1515	5.20 [b]	7.5
Plantain 6L	1549	6.20 [a]	6.7
SEM	171.3	0.18	0.38
P value	0.29	<0.001	0.17

4.3.2 Nutritive value of fresh and wilted plantain during autumn

Increasing the stage of maturity of plantain in autumn improved the ME content of the forage used for silage (Table 4.4). Delaying harvest at each leaf stage resulted in increasing DM%, OM%, WSC% and ADF%, while, at the same time, reducing CP% (Table 4.4). There was no reproductive material at this time with leafy plants contributing to the low DM (below 16%). These low values were increased somewhat after wilting, though two to three days post harvest the DM% remained lower than 25%.., Fibre was relatively low for all treatments with highest NDF at 5L but, after wilting, the NDF was similar for 4L and 5L. ADF was similar at 5L and 6L and was lowest at 4L. Digestibility in

general was high, though the lowest value was observed at 4L and, combined with the low OM content, this resulted in lower ME compared with 5L and 6L.

Two of the three secondary metabolites of fresh plantain increased between the early stage of maturity and the late stage of maturity. Aucubin at 4L was higher at 27.3% compared to 6L. However, catalpol and acteoside were higher at 6L compared to 4L. The differences were 46.6% and 28.9% for catalpol and acteoside, respectively. Among the secondary metabolites, acteoside had the highest proportion in fresh plantain.

Table 4.4. Summary of nutritive values of plantain before and 24 hours after harvest (%DM)

Kind of nutrition	4-leaf appearance		5-leaf appearance		6-leaf appearance	
	Fresh	Wilted	Fresh	Wilted	Fresh	Wilted
Dry matter (%)	13.4	17.8	12.2	19.4	15.2	22.1
Organic matter (%)	77.5	78.4	84.7	80.5	83.7	85.5
Neutral detergent fibre (%)	15.8	19.9	20.8	19.9	13.37	17.6
Acid detergent fibre (%)	12.6	13.2	13.6	15.0	13.8	15.3
Crude protein (%)	19.4	21.2	19.4	19.3	16.7	16.5
Water soluble carbohydrate (%)	9.46	8.15	10.4	9.13	11.78	10.2
DM digestibility (%)	77.8	78.5	82.3	78.6	81.6	83.1
OM digestibility (%)	89.8	88.5	91.5	89.4	91.6	91.6
Digestible OM in the DM (%)	69.6	69.4	77.5	71.9	76.6	78.3
Metabolisable energy (MJ/kg DM)	11.1	11.1	12.4	11.5	12.3	12.5
Aucubin (mg/g DM)	0.28	-	-	-	0.22	-
Catalpol (mg/g DM)	2.36	-	-	-	3.46	-
Acteoside (mg/g DM)	28.4	-	-	-	36.6	-

4.3.3 Fermentation characteristics of plantain silage

Upon opening the silos at each treatment date, visible cues were similar as those observed in Chapter 3. The silages were very dark, mould was evident on the outside of the bag, but quite low at <15% of the outer surface and centralised near the opening of the bags (Plate 4.1). The silage smelt sweet and well fermented. However, in general, the fermentation characteristics of the silage were poor (Table 4.5). The pH of plantain silage at all stages of maturity was high (pH > 5.0); only 4L the pH was less than 5.0. However, with longer storage, the pH decreased, although the value was still higher than five and there were no interactions between the stage of maturity and storage duration. The BC was very high (above 300 meq./100 g DM) and there was an interaction between the stage of maturity and the duration of storage, where an increase in maturity stage and storage duration had a positive effect on the BC value.

Total VFA decreased with the increased stage of maturity but decreased with prolonged storage duration. The lactate percentage diminished with the delay of harvesting but was higher with the longer storage period. However, the percentage was very low, below 1.5%. This also occurred in the acetic acid content at the mature stage. There was no effect of storage duration on the acetic acid content. Butyric acid, which was also a sign of low quality of silage, was very low at all stages of maturity and storage duration, below 0.2%.

Propionic acid was low, at not more than 0.12%. The lowest propionic acid was at 5L at less than 0.1%. The percentage of mould was affected by the stage of maturity where the mould percentage at 4L was the highest than the other treatments, but there were no significant differences at the different storage durations.

a).

b).

c).

d).

Plate 4.2.Performance of mini silo of plantain in autumn harvested (a), plantain 4L silage (b), plantain 5L silage (c), plantain 6L silage (d), stored for 90 days.

Table 4.5. Fermentation characteristics of plantain four-leaf appearance (4L), five-leaf appearance (5L), six-leaf appearance (6L) silage (% DM).

Treatment	Storage duration (days)	pH	BC (meq./100g DM)	Total VFA (%)	Acetic acid (%)	Butyric acid (%)	Lactic acid (%)	Propionic acid (%)	NH_3-N (% of total N)	Visual assessment of mould (%)
4L	90	5.37	450 ^{cde}	2.17 ^{b}	1.43 ^{bc}	0.06	0.59	0.09	3.94	17.0
	120	4.89	514 ^{bc}	3.39 ^{a}	1.75 ^{b}	0.12	1.43	0.09	4.76	13.0
	150	4.91	743 ^{a}	4.32 ^{a}	2.47 ^{a}	0.11	1.57	0.17	5.07	19.0
	180	4.71	597 ^{b}	4.13 ^{a}	1.75 ^{b}	0.16	2.1	0.12	4.76	15.0
5L	90	5.88	272 ^{f}	1.25 ^{b}	0.93 ^{cd}	0.00	0.21	0.1	4.69	13.8
	120	5.56	348 ^{def}	1.84 ^{b}	1.14 ^{bcd}	0.01	0.63	0.07	4.33	13.4
	150	5.69	406 ^{cdef}	1.74 ^{b}	1.10 ^{bcd}	0.02	0.55	0.08	4.21	13.0
	180	5.6	355 ^{def}	1.86 ^{b}	0.87 ^{cd}	0.48	0.41	0.09	4.07	15.0
6L	90	6.14	320 ^{ef}	1.18 ^{b}	0.99 ^{cd}	0.00	0.09	0.1	5.18	12.0
	120	6.0	447 ^{cde}	1.39 ^{b}	1.11 ^{bcd}	0.02	0.14	0.12	5.38	13.0
	150	6.2	394 ^{cdef}	1.03 ^{b}	0.71 ^{d}	0.08	0.13	0.12	5.38	13.0
	180	5.8	482 ^{bcd}	1.87 ^{b}	1.22 ^{bcd}	0.22	0.27	0.17	4.85	15.0
SEM		0.28	31.8	0.28	0.16	0.09	0.25	0.02	0.31	1.29
Regrowth		<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.01	<0.001	0.01
Storage		0.02	<0.001	< 0.001	0.14	0.74	0.025	0.1	0.59	0.26
Regrowth x Storage		0.78	0.002	0.006	0.002	0.21	0.15	0.11	0.14	0.09

4.3.4 Nutritive value of plantain silage

The moisture content of the silage was greater than observed in the raw silage material. The DM% was very low, below 20%, at all stages of maturity (Figure 4.2). The DM% was affected by maturity stage and storage duration. In terms of organic matter, the more advanced the stage of maturity the greater the OM%.A similar trend was noted for NDF, which increased from 4L and 6L and was highest at 5L. Overall, the NDF was low, below 30%, and the ADF was below 23%. Crude protein was high, above 20%, and there was no interaction between the maturity stage and storage duration. The increasing storage duration had no effect on the concentration of the crude protein.

The ME improved with the stage of maturity owing to the greater OM content and digestibility at 6L compared with 4L and 5L. The ME of 6L was lower in silage than in the fresh or wilted stage but remained highest, above 10 MJ/kg DM, whereas both 4L and 5L were below 9.5 MJ/kg. The longer storage duration reduced the DOMD and ME, which where highest at 90 days of storage duration.

For the secondary metabolites, all concentrations were considerably lower compared with the fresh forage. There was no aucubin present after the plantain was ensiled. Catalpol had disappeared from 4L in 90 and 180 days but there was a tiny amount remaining at 6L at days 90 and 180 although there were no significant differences among the treatments. For acteoside, the remaining concentrations were very low but remained stable until 180 days of storage, with the highest concentration found at 6L after 90 days of storage.

Table 4.6. Nutritive value of plantain four-leaf appearance(4L), five-leaf appearance(5L) and six-leaf appearance (6L) silage during autumn 2017.

	OM (%)	NDF (%)	ADF (%)	CP (%)	DMD (%)	DOMD (%)	ME (MJ ME/kg DM	Aucubin (mg/g)	Catalpol (mg/g)	Acteoside (mg/g)
Regrowth stage										
4L	73.2 [a]	27.4 [a]	19.7 [a]	23.0 [a]	71.5 [a]	58.2 [a]	9.31 [b]	0	0	0.08
5L	77.3 [b]	28.8 [b]	22.2 [b]	21.5 [b]	72.4 [a]	61.1 [a]	9.77 [b]	-	-	-
6L	79.9 [c]	26.2 [a]	21.8 [b]	21.4 [b]	76.0 [b]	65.2 [b]	10.4 [a]	0.05	0	0.09
SEM	0.81	0.35	0.34	0.28	0.75	0.92	0.15	0.02	-	0.004
P value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.06	-	0.11
Storage duration										
90 days	78.9	27.6	20.9	22.2	74.8	64.1 [b]	10.3 [a]	0.014	0	0.1 [a]
120 days	75.1	27.0	20.9	21.6	72.0	59.7 [a]	9.55 [b]	-	-	-
150 days	76.8	27.4	21.3	22.3	73.6	61.4 [ab]	9.82 [ab]	-	-	-
180 days	76.4	27.8	21.8	21.9	72.8	60.8 [a]	9.73 [b]	0.039	0	0.08[b]
SEM	0.94	0.40	0.395	0.28	0.86	1.1	0.17	0.02	-	0.04
P value	0.05	0.55	0.31	0.45	0.15	0.03	0.03	0.31	-	0.03
Regrowth x storage	0.76	0.73	0.80	0.23	1.490	0.52	0.52	0.31	-	0.099

*The different notation in the same column shows significant results (P<0.05). DM=Dry matter, OM=Organic matter, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre, CP=Crude protein, DMD = Dry matter Digestibility, DOMD=Digestible Organic Matter in Dry Matter.

There was an interaction between treatment and storage for DM percentage of plantain silage made in autumn (Figure 4.2.). Dry matter was highest for 5L and similar for 4L and 6L. Generally, DM% increased with storage duration except at 150 days for 4L and 180 days for 6L which showed lower DM%. There is no liquid produced in the mini silo.

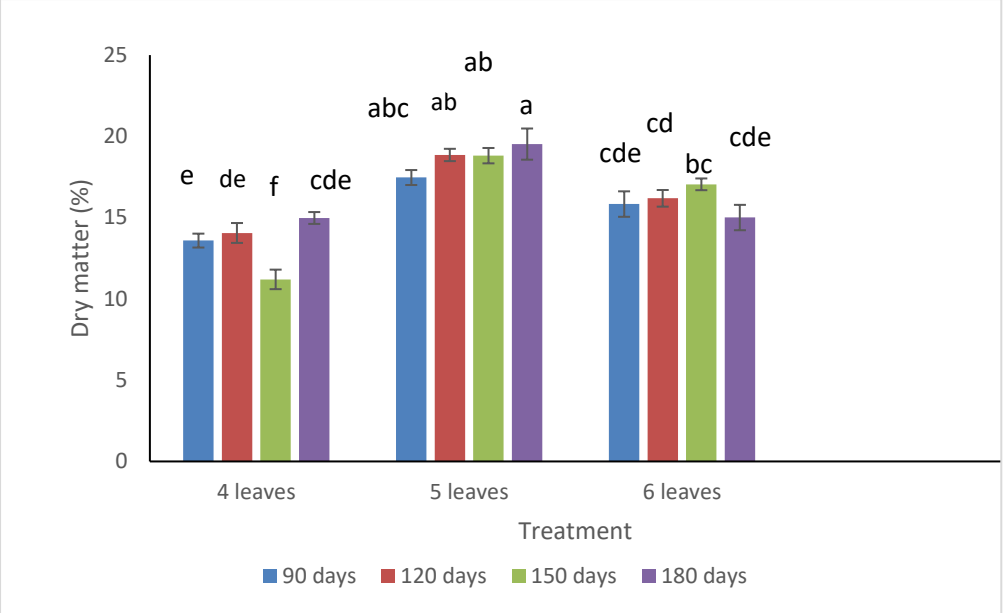

Figure 4.2. DM percentage of plantain silage made in autumn

4.4 Discussion

The hypothesis for the present study was that in autumn, the quality of the silage would be good due to more leaf relative to stem and that advancing the stage of maturity of the plantain may not reduce nutritive value if leafy material is maintained. In this study, advancing the stage of maturity from the 4L to 6L appearance showed a negative relationship between maturity and silage quality in terms of fermentation characteristics, but it showed a positive relationship of the nutritive value. Moreover, plantain silage produced in autumn can be stored for up 180 days without impacting the nutritional content.

4.4.1 Fermentation characteristics of plantain silage

The pH of plantain silage produced in autumn was relatively high, ranging from 5.0 to 6.0, compared with spring silage that had pH ranging from 4.9 to 5.5 (Chapter 3). The high pH which continued to decline from 90 to 180 days storage indicates that the rate of fermentation was slow (Pahlow et al., 2003b). This was also evident because the pH decreased with prolonged storage as the lactic acid was still increasing (Table 4.4).

A slow or incomplete fermentation phase can occur for a number of reasons, and often these variables are interrelated. A high buffering capacity, insufficient fermentable energy for LAB, or insufficient N source for LAB or an imbalance in protein and energy can all contribute to poor fermentation. Similarly, plant properties such as antimicrobial secondary compounds may also reduce LAB activity during fermentation. The buffering capacities of these silages were very high and higher than those of silage produced during the late spring (Chapter 3). This was because plantain harvested during autumn was in the vegetative stage and a large proportion of the buffering comes from cellular contents of the leaf stated that the buffering capacity of plants in a vegetative stage had higher BC compared to those produced in a generative stage. During vegetative stage, plant has high protein content that contributes to the high BC (Playne & McDonald, 1966). Further stated, side chain of carboxyl group of glutamic and aspartic acids are responsible to BC. A high buffering capacity is not beneficial for aerobic stability because it can reduce the anaerobic stability and that in turn, will reduce the aerobic stability of silage when exposed to the air (Pahlow *et al*. 2003).

Lactic acid produced in this study was very low indicated by the high pH. Although the WSC, which acted as an energy source for LAB, was higher compared to those in spring plantain silage, the low lactic acid production may have been caused by high secondary metabolite production during autumn that inhabited the degradation of protein (Tamura & Nishibe, 2002). Crude protein should be broken down into amino acids, peptides and small amounts of NH_3 (Harrison, Blauwiekel, & Stokes, 1994). Lactic acid bacteria need amino acids, vitamins and energy to grow, which was usually supplied by WSC (Gibbs *et al*. 1950; Pahlow, Muck, Driehuis, Elferink, & Spoelstra, 2003).

The ammonia N of silage produced in autumn was close to 5%, higher than previous observations of ammonia N of less than 1% in spring harvest plantain (Chapter 3). This may because of the higher concentration of the crude protein in silage produced during autumn. But the percentage of ammonia N was still categorised as good for silage as it should not have a high NH_3-N of more than 8% (Kung Jr *et al*. 2018). During the ensiling process, proteolysis occurs extensively to produce non-protein nitrogen, but in well preserved silage this N is present as amino acids and peptides (Betteridge & Sedcole, 1982). With prolonged storage there were no differences in the NH_3-N concentration, and this means that silage produced in autumn can be stored for 180 days with little degradation.

Acetic acid production had similar values for all storage durations. The increased storage duration did not change the percentage of acetic acid. This means that there was no increase in anaerobic deterioration in these silages until 180 days of storage. Acetate is needed for antifungal compound (Bernardes *et al.* 2018).

Similarly for propionic acid, the higher contents were at 4L and 6L and the lowest was at 5L although the difference was only very small. The contents of propionic acid did not change with the changes of storage duration (Weinberg *et al*. 1995). It can be expected that the chance for aerobic stability was not different under all storage durations.

Although this plantain silage had a high pH, the aroma upon the opening of the bag was not rancid. This was supported by the very low butyric acid content, at below 0.5%. Management influences the characteristics of silage fermentation. The stage of maturity, known as a part of management, has a major influence on making silage (Buxton, 1996). The results of Chapter 3 showed that a delayed harvest increased the mould percentage of the silage. However, in this study, the percentage of mould was higher at the early regrowth stage. This may because at the early stage of maturity the moisture content was the highest, accounting for 86.5% of the fresh weight. Moisture level is one of the factors that influences mould growth (Driehuis, 2013). However, with prolonging storage, the mould growth was similar at all storage durations. This was due to the moisture content of plantain silage not changing with prolonged storage. The possible reason for this was the oxygen that entered the mini silo was at a minimum because there were no seed heads in the plantain silage as they were not resilient as those occurring in the spring silage because more seed heads were produced in the spring.

4.4.2 Nutritive value of plantain silage

The nutritive value of this silage declined considerably between harvest, wilting and ensiling. At the time of harvest, ME content was over 12 MJ ME/kg DM, but the majority of the silage had an ME of less than 10 MJ ME/kg DM. The longer storage duration had almost no effect on the nutritive value of the silage so the reduction in quality is expected to have occurred during the aerobic, lag or fermentation phase.. Most likely this loss was because of the degradability of nutrients by microorganism during anaerobic fermentation (Buckmaster, Rotz, & Muck, 1989). The chemical composition of the raw material will have had a pronounced effect on lack of retention of nutritive value.

One of the important features of silage quality is the moisture content. Increasing the DM content through wilting is required for silage production, although wilting has a minor effect on the nutritive values of the silage. The low air temperatures during harvest and wilting resulted in low DM% even up to three days after harvest. Ideally, the DM of the raw material of silage should be around 30% for making good silage (Moran, 2005). The DM in this trial was below the desired target of 30% because at this time could not be dried more because of the high moisture present during the wilting period.

Variation between chemical composition at the time of harvest compared with the raw silage highlight some issues in which analytical results describe forage chemistry. In the original wilted forage, the fibre and protein fraction accounted for roughly 50% of the OM, but after ensiling they accounted for 65-70% of the OM. The majority of the unexplained dry matter is made up of non-structural carbohydrates which may or may not act as an energy substrate for LAB. Using conventional testing methods the measured concentrations of WSC were relatively low at around 9% of the DM. Jiang, Bryant, Jiao, & Tung (2019) recognised different sugar types in plantain herbage, namely sorbitol which made up the soluble fraction. Similarly, pectin and mucilage carbohydrates account for remaining NSC which are not resistant to neutral or acid detergents. The slow fermentation observed in these silo's may arise from slow availability of alternative carbohydrates or from low abundance of the types of bacteria which use alternative sugars sources for energy.

Fibre concentration increased from harvest to ensiling. Initially the harvested plantain had relatively high digestibility especially at 6L, which was greater than at 4L and 5L. This too was unusual because most studies show decreasing DMD with advancing maturity. This is probably due to increased number of mature leaves (Table 4.2) with greater cell contents to cell wall ratio as reflected by the low NDF in 6L compared with earlier harvests. Overall, fibre was low in all treatments and this lower NDF is probably because autumn has low temperatures that reduce the lignification of plantain (Van Soest, 1983) and result in a corresponding improvement in digestibility (Kolver, Roche, Miller, & Densley, 2001).

There was a low fibre content in the plantain because there were no seed heads produced. Consequently, it was expected that plantain ensiled in autumn would produce good quality of silage. The ADF content was low (below 16%) at all stages of maturity and the content increased with the increasing maturity stage. Conversely, the NDF content decreased with the delayed

harvest. The possible reason is that autumn has low temperatures that decrease lignification and so improve forage digestibility (Van Soest, 1983) and a corresponding improvement in digestibility. DM digestibility increased with the increasing maturity stage as well as ME.

The crude protein of this plantain silage was higher than the requirements, at above 20%, as the target for high quality of silage is more than 16% (Howse *et al*. 1996). The high protein concentration in silage was beneficial for microbial growth in the rumen (Bernardes *et al*. 2018) especially with the higher concentrations of peptide N (Nsereko & Rook, 2000, Choi *et al*. 2002). Crude protein was also not affected by the duration of storage. Antimicrobial compounds, in plantain, such as acteoside, might be a reason for this low proteolysis (Isselstein, 1993 *cited in* Stewart, 1996). Plant secondary metabolites were degraded and disappeared from forage during ensiling. These results show the repeatability of observations from Chapter 3.

4.4.3 Other considerations

Often farmers will ensile pasture with the goal of increasing the yield of harvested silage as a way of reducing costs of production. In this study, the DM production of autumn grown plantain was low at all stages of maturity, with an average of 1400 kg DM/ha in the above ground parts (Table 4.1). The lack of maturity on yield is the product of similarities in the yield components, such as shoots per plant and yield per shoot. The areas were assumed to be balanced for plant density. The similarity in yield might be due to the age of the pasture because the experimental site was three years old; thus, the distribution of plants was variable and the large standard errors reflected the uneven distribution of the plantain. Numerically, there was an increase of 35% in yield between 4L and the other stages, which was likely driven by the increased appearance of leaves but, given the time of the year, the lack of above ground assimilates may also be the result of the increasing partitioning below ground. If secondary metabolites in forage plantain are responsible for variation in animal N losses then using plantain silage to achieve reductions in N loss may not provide the desired outcomes. Having said that, plantain silage from autumn harvest has high protein content which is a useful for an animal supplement.

4.5 Conclusion

Fermentative characteristic of plantain at the early stage of maturity harvested in autumn resulted in better total VFA and lactic acid content, however mould percentage was higher compared to that of plantain harvested at the late stage of maturity (6L). The nutritive value did not change with storage duration, still plantain 6L silage was better compared with other stages of maturity in terms of organic matter and NDF percentage. Besides, the digestibility and ME of plantain 6L was also better compared to that of other stages of maturity. However, secondary metabolite was degraded during ensiling that might affect animal performance. The better quality of plantain silage made from autumn trial might have advantages to produce this silage for a commercial size that will benefit to animal production.

Chapter 5

Effect of fertilisers and additives to the ensiled plantain

5.1 Introduction

The quality of the ensiled feed is determined by the raw material of silage i.e., the plant/pasture, and this, in turn, is influenced by management. For example, the use of commercial products such as fertilizer before harvest or additives after harvest are management tools to increase plant quality and quantity (Sameni & Soleimani, 2007). Application of inorganic N fertilizer to conventional grass pastures is used to improve herbage yield (Buxton & O'Kiely, 2003), and also improve quality through increased herbage CP concentration (Keady & O'Kiely, 1996). Similarly, in plantain (*Plantago lanceolata*) positive responses to N fertiliser have been observed (Lambers *et al*. 1981).

Other fertilizers that are commonly used on the farm are phosphorus and potassium, often applied in combination with nitrogen (e.g. as NPK). Fertilization with NPK has shown to increase total soil antioxidant capability, which may also improve plant antioxidant capability (Skwaryło-Bednarz & Krzepiłko, 2009), making the plant less susceptible to disease. As plantain is susceptible to a wide range of fungal and bacterial diseases (Stewart, 1996), it is hypothesised that reduction of disease, as a result of fertiliser treatment, will improve the yield and nutritive quality of its silage.

In addition to fertilizer, additives are commonly used to improve silage quality post harvest. There are many additives commercially available for silage, such as enzymes, sugars, and inoculants (Bolsen *et al*. 1996). Cell wall degrading enzymes have shown to improve digestibility characteristics of maize silage due to the reduction of fibre content (Colombatto, Mould, Bhat, Phipps, & Owen, 2004). Furthermore, enzymes break down plant cell wall which, in turn, make sugar more available which improves the fermentation profile (Muck & Shinners, 2001). The value of additives have been demonstrated using enzymes with hay crop silage which resulted in improved DM intake of ruminants consuming this silage (Chen, Stokes, & Wallace, 1994).

Because the primary substrate of bacteria fermenters are simple sugars, another common additive is molasses. Molasses is the by-product of sugar extraction and contains about 20% sucrose, 20% reducing sugars, 10% ash, 20% nonorganic sugars, and 20% water (CAS No. 68476-78-8). Because molasses is rich in sugar content, it is advantageous for improving fermentation quality, especially in low DM and low WSC grass, and tropical forages (Bolsen *et al*. 1996). The effect of adding molasses

is to support lactic acid producing bacteria and increase the subsequent lactic acid percentage, decrease butyric acid and improve stability of the silage product (Ni *et al*. 2017).

Finally, in much the same way as it is beneficial to improve the growth of lactic acid bacteria (LAB) by providing energy in the form of sugar, some users will choose to inoculate silage directly with live bacteria. The purpose of the inoculants application is to increase the rate of fermentation in silo (R. Muck & Shinners, 2001). A rapid rate of fermentation and lactic acid production is needed to achieve low pH to stabilise the silage and maintain the nutritional value (Muck, 2002).

Previous results showed that water soluble carbohydrate in plantain harvested in spring was relatively low (Chapter 3) compared with published values for ryegrass silages. Consequently, fertilizer and additive may improve silage quality by increasing the sugar content and fermentation characteristics of plantain (McDonald *et al*. 1991; Muck, 2002). Currently, there is no information on the influence of fertilizer and additives on the quality of plantain silage. The objectives of this study were to investigate the effect of both fertilizer and additives on the fermentation characteristics and nutritive value of plantain silage. The hypothesis of this study is that fertilizer and additives will enhance the fermentation characteristics and nutritive value of plantain silage.

5.2 Materials and methods

5.2.1 Experimental Site and Design

This study was conducted at the Lincoln University Research Dairy Farm (LURDF) Canterbury, New Zealand (43°38'S, 172°27'E) in late spring 2016 (14 November – 28 December 2016). The experimental area (plantain pasture) was the same as described in Chapter 3. The herbage mass was previously managed by rotational grazing with dairy cows.

The experiment was a 3 x 4 split plot design with four replicates (Figure 5.1.). Fertilizer was the whole plot treatment and additive was the subplot treatment. The fertiliser treatments were nitrogen only at 20 N/ha as urea (20N); nitrogen, phosphorus and potassium at 20:1:15 kg/ha (20NPK) or nitrogen, phosphorus and potassium at 40:1:15 kg/ha (40NPK). The additive treatments were no additives (CON), cellulose enzyme (ENZ): Biosil, (BIO); and molasses (MOL).

On 14 November 2016, the entire area was mown to a height of 6 cm and treatment plots (2 x 5 m) were marked out using wooden pegs. The fertilizer treatments were weighed out to meet the application rate targets (43 g urea for 20N, 100 g of NPK for 20NPK and 43 g urea + 100 g NPK/plot for 40NPK, respectively) and applied evenly by hand to designated plots.

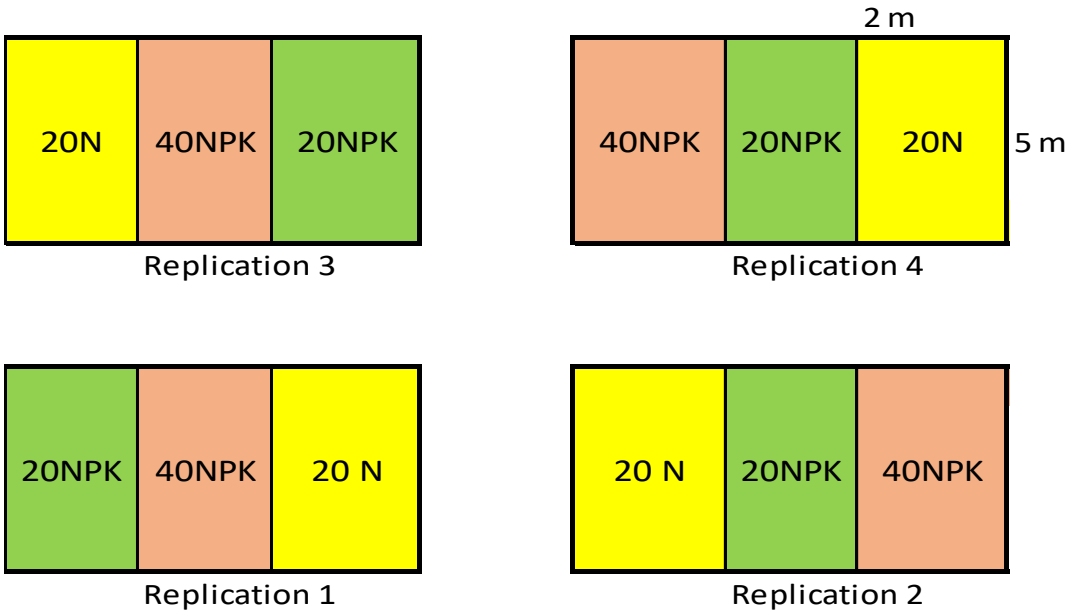

Figure 5.1. Map of experiment

5.2.2 Measurements

By late December 2016, plantain had reached the six-leaf stage after approximately 400 growing degree days (GDD). Ten plants in each plot were randomly selected and the numbers of new leaves were counted, as were the number of seed heads and shoots per plant. To determine the effect of treatment on yield, the DM production of plantain in each treatment was calculated by harvesting replicated (n=3) quadrats (0.2 m^2) by cutting to ground level in each plot with the electric handpiece. Overall, 36 of quadrat cuts were taken from the experimental area. All unsown species were removed leaving plantain only for DM determination. Then the samples were oven- dried at 60°C for 48 hours.

At 1600 h, on 27 December, all plots were mown to 6 cm height using electric hand shears and left on the ground to wilt. A subsample which was from composited samples (150 g FW) of fresh herbage from the mown area was collected and processed immediately after cutting to determine the quality of fresh forage. The timing of ensiling was described in Chapter 3. Basically, forage was considered ready to ensile once it passed the 'squeeze test'. The DM of wilted plantain was confirmed by oven drying a 50 g FW of composited samples at 60°C for 48 hours. Nutritive content was determined on approximately 150 g of fresh, and wilted plantain which was freeze-dried and ground for further analysis.

All the mown plantain from each plot was divided into four, and ensiled by using enzyme, biosil and molasses, respectively and the other was as a control. Each plot replicates representing one silo (n=4

silos/treatment). Any unsown species were removed, and discarded to ensure that only plantain was ensiled. The wilted plantain as a control (approximately 500 g FW) was immediately pressed into plastic bags as described in Chapter 3. Additive treatments were sprayed evenly to the wilted plantain in the determined plastic silo bag by using a syringe. The silages were stored for 180 days in a black bin.

Additives for ENZ involved diluting 1.5 g of cellulase enzyme in 20 ml of water which was added at 1.7 ml per 500 g of wilted plantain (Tengerdy *et al*. 1991). For the BIO treatment, 20 mg of commercial product Biosil (a combination of bacteria (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Propionibacterium acidipropionici* and enzymes (cellulase, hemicellulase, amylase, and beta-glucanase) was suspended in 0.4ml of tap water and allowed to stand for 10 minutes. The suspension was diluted further to 20 ml and 2 ml of the solution was applied to 500 g of wilted plantain. Molasses was added to wilted plantain at 10 ml per 500 g as recommended by Bolsen *et al*. (1996).

5.2.3 Herbage analysis

After 180 days, silos were removed, and fermentation measurements were carried out similar to those described in Chapter 3. Briefly, mould percentage was estimated from visual scoring the percentage of the mould growth on the surface area of the silo. Dry matter percentage was determined by weighing approximately 50 g FW sample and oven drying at 60°C for 48 hours. Botanical morphology was measured by separating a 25 g silage sample into stem and leaf which was then oven dried for 48 hours at 60°C. The pH and buffering capacity (BC) followed the procedure stated by Playne & McDonald (1966). Volatile fatty acids (VFA) except lactic acid of samples were analyzed by using Gas Chromatography (GC-2010, Shimadzu, Japan), whereas lactic acid and NH3-N were measured by using Randox (Rx Daytona, UK).

Nutritive characteristics of fresh, wilted and ensiled plantain were determined by wet chemistry using freeze-dried samples that had been milled through a 1 mm screen centrifugal rotor mill (Retsch-ZM, Haan, Germany). Acid Detergent Fibre (ADF) was determined gravimetrically following the procedure stated by AOAC (1990). Neutral detergent fibre (NDF) was determined by (Van Soest et al., 1991). Crude protein (CP) was based on total N concentration which is measured using the elementor analyzer Vario Max CN analyzer. Water soluble carbohydrate (WSC) was analyzed using the colorimetric method with anthrone reagent described by Pollock and Jones (1979) and for this experiment, WSC analysis was only for fresh and wilted plantain. Dry matter digestibility (DMD) was

based on the procedure described by Clarke *et al*. (1982). Metabolisable Energy(ME) was calculated based on CSIRO (2007); $MJME\ /\ kgDM = 0.16 \times DOMD$

5.2.4 Meteorological data

Meteorological data was taken from the National Climate Database (http://cliflo.niwa.co.nz). The growing degree day (GDD) was calculated using mean temperature and thermal requirements of plantain where:

$$GDD = \frac{\text{Temperature maximum + temperature minimum}}{2} - \text{base temperature}$$

The base temperature used for plantain was 5°C based on Powell et al. (2007)

5.2.5 Statistical analysis

Yield and nutritive data of fresh plantain were analyzed using one way ANOVA of the statistical package GenStat version 18 (VSN International Ltd, 2015) using fertiliser as the fixed term and replicate as the random term in the model. To compare the effect of fertilizer, additive, and their interaction on silage variates, the split-plot design model in GenStat version 18 (VSN International, 2015) was used. Where fertilizer was the whole plot term and additive as the split plot term and silo replicate as the random term.

5.3 Results

5.3.1 Climate condition

The temperature during the regrowth period had an average maximum of 19.6^{o} C and an average minimum of 9.3^{o}C. The average day length was 14.2 hours and average sunshine hours were 5.34 hours/day. The total accumulated GDD was 425 for plantain with 5^{o}C as a base temperature (Powell et al., 2007). At the time of harvest, the temperature ranged between 19.4^{o}C and 22.2^{o}C while the range in temperature throughout the remaining wilting period (around 24 hours) was 9 - 17.4^{o}C (Figure 5.2).

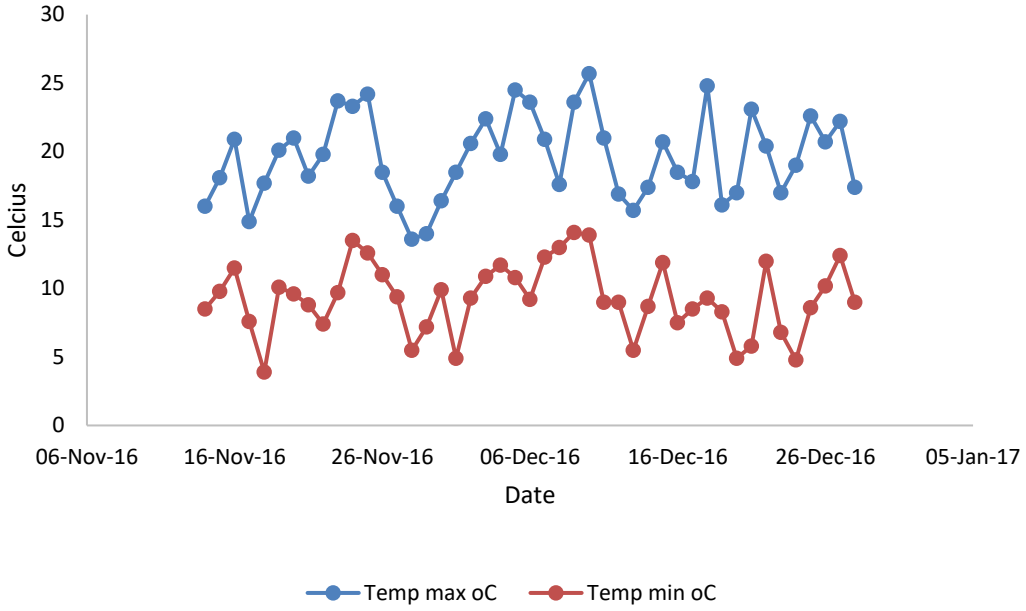

Figure 5.2. Air temperature at the experimental site between 14 November and 28 December 2016.

5.3.2 Pre-Ensiling

Overall the results showed that with increasing ammonium-N-based fertilizer (NPK), the DM production of plantain was increased ($P<0.05$, Table. 5.1). The plantain with urea N fertiliser had intermediate DM yield compared to 20NPK and 40NPK. At 40NPK, the yield increase was associated with greater leaf number on the main shoot (>6) compared with a low number of leaves (<5) at either fertiliser applied at 20 kg N/ha. However, there was no difference between fertiliser treatments for seed head or shoot number, though the urea-treated plantain had a tendency for fewer seedheads and but more stem content than the fertilisers which included P and K.

Table 5.1. The influence of different fertilizers to the plantain yield, shoot number, leaf number, number of seedhead and stem content.

Treatment	Yield (kg DM/ha)	Shoot number/plant (shoots)	Leaf number/shoot (leaves)	Number of seedhead/plant (seedhead)	Stem percentage (% of DM)
20 N	1720 [ab]	10.4	4.49 [a]	19.3	84.9
20NPK	1519 [a]	10.9	5.10 [a]	21.5	78.7
40NPK	1829 [b]	10.2	6.25 [b]	24.6	78.8
SEM	69.2	0.46	0.20	1.26	2.12
P value	0.049	0.55	0.002	0.064	0.07

Note : Different notation in the same column show significant result. Where DM is dry matter
20N=20 kg/ha of Nitrogen, 20NPK = nitrogen, phosphorus and potassium at 20:1:15 kg/ha;
40NPK = nitrogen, phosphorus and potassium at 40:1:15 kg/ha.

5.3.3 Fresh and wilted plantain

It can be seen in table 5.2 that there was no effect of different level of fertilizer on the nutritive value of fresh plantain. The DM % of wilted plantain was in the desired range of DM at ensiling (>30%). ADF increased significantly at wilted plantain where 40NPK had the highest. However, there was no reduction in DMD and OMD of wilted plantain. Insignificant result was also found in DOMD and ME. Wilting boosted the OM value of plantain ($P<0.01$), where the highest was at 40NPK. However, wilting did not affect CP and WSC percentage of plantain. Ash content was also not affected by wilting.

Table 5.2. Effect fertilizer and wilting on the nutritional characteristics (% of DM) and metabolisable energy (ME MJ/kg DM) of plantain.

	DM (%)	ADF (%)	NDF (%)	OM (%)	CP (%)	WSC (%)	Ash (%)	DMD (%)	OMD (%)	DOMD (%)	ME (MJ ME/kg DM
Fresh											
20N	26.0	26.6	41.5	91.4	10.2	7.35	8.72	69.7	74.0	69.6	11.1
20NPK	23.7	26.8	41.9	91.2	10.2	7.25	8.61	68.8	73.0	68.8	11.0
40NPK	24.4	26.7	41.8	91.6	10.7	7.66	8.59	68.9	73.7	69.2	11.1
SEM	1.01	0.37	0.66	0.24	0.26	0.25	0.28	0.59	0.80	0.68	0.11
P value	0.31	0.97	0.93	0.62	0.33	0.52	0.87	0.52	0.64	0.68	0.68
Wilted											
20N	33.7	28.1[b]	42.6	92.3 [b]	11.0	6.58	8.08	68.8	72.6	69.3	11.1
20NPK	31.9	27.6 [b]	42.5	92.3 [b]	11.3	7.44	8.46	68.5	73.1	68.9	11.0
40NPK	30.9	30.1[a]	46.8	93.4 [a]	9.89	6.75	8.12	64.5	68.2	65.5	10.5
SEM	1.2	0.54	1.15	0.22	0.42	0.51	0.46	1.14	1.30	1.09	0.18
P value	0.32	0.04	0.06	0.02	0.12	0.50	0.68	0.06	0.07	0.09	0.09

*Different notation in the same column showed significant result (P<0.05)
Where 20N=20 kg/ha of Nitrogen, 20NPK = nitrogen, phosphorus and potassium at 20:1:15 kg/ha;
40NPK = nitrogen, phosphorus and potassium at 40:1:15 kg/ha.
Dry Matter (DM), Organic Matter (OM), Crude protein (CP), Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), Digestible Organic Matter in Dry matter and Metabolisable Energy

5.3.4 Fermentation characteristics of plantain silage

The fermentation characteristics of the mini silos are presented in Table 5.3. There was no interaction between fertilizer and additive for any variables except lactic acid (P = 0.03) and NH3 N percentage (P = 0.017). The pH of all silos was relatively high, at more than 5.5. However, the main effects for additive showed that any of the additives reduced pH compared to CON. Higher N fertilizer also resulted in lower pH. Average BC was 273 meq/100 g DM, which was increased by the inclusion of phosphate and potassium and reduced by the addition of MOL. The average total VFA was almost 1%, which was raised only by fertilizer. NH3-N was very low at an average of below 2%, and it was increased with the increase of fertiliser, and it reduced by MOL. Butyric acid was no different among treatments, and the percentage was very low at 0.08%. Propionic acid was affected by fertilizer where the higher application of fertilizer resulted in a higher content of propionic acid. The average value of propionic acid was 0.04 %. Mould percentage was close to 50% but reduced by nitrogen fertilizer and MOL.

a).

b).

c}.

d).

e).

Plate 5.3.Mini silo (a), performance of silage without additives (b), with Biosil (c), with enzyme (d) and molasses (e).

Table 5.3. Effect of additives and fertiliser on plantain silage pH, buffering capacity (meq/100gDM), total volatile fatty acid, acetic acid, butyric acid, propionic acid (%), mould percentage (%).

	pH	Buffering capacity	Total VFA	Acetic acid	Butyric acid	Propionic acid	Mould percentage
Fertiliser							
20N	5.88 [b]	234.7 [b]	0.67 [b]	0.19 [b]	0.03	0.02 [b]	53.7 [a]
20NPK	5.94 [b]	291.9 [a]	1.04 [a]	0.31 [ab]	0.05	0.05 [a]	54.7 [a]
40NPK	5.55 [a]	292.4 [a]	1.25 [a]	0.39 [a]	0.15	0.05 [a]	41.9 [b]
SEM	0.14	12.82	0.08	0.043	0.04	0.01	2.9
P Value	0.02	0.004	<0.001	0.008	0.08	<0.001	0.005
Additives							
Control	6.20 [c]	285.7 [ab]	0.83	0.35	0.07	0.04	50.4 [ab]
Biosil	5.55 [ab]	260.2 [ab]	0.97	0.23	0.05	0	48.8 [ab]
Enzyme	5.87 [b]	314.1 [a]	1.17	0.38	0.17	0.05	58.3 [a]
Molasses	5.51 [a]	232.1 [b]	0.97	0.24	0.03	0.03	42.9 [b]
SEM	0.11	14.8	0.09	0.05	0.04	0.01	3.31
P value	<0.001	0.003	0.11	0.09	0.16	0.24	0.02
Fertilizer x Additives	0.663	0.573	0.342	0.482	0.512	0.939	0.638

*Different notation in the same column showed significant result (P<0.05)
Where SEM=standard error of means: Where 20N=20 kg/ha of Nitrogen, 20NPK = nitrogen, phosphorus and potassium at 20:1:15 kg/ha; 40NPK = nitrogen, phosphorus and potassium at 40:1:15 kg/ha.

The interaction between fertilizer and additives for ammonia content of plantain silage (Figure 5.3.) showed that ammonia concentrations were lowest for silo's which had received 20N fertiliser only. But, depending on the additive, ammonia concentrations increased or decreased relative to control silo.

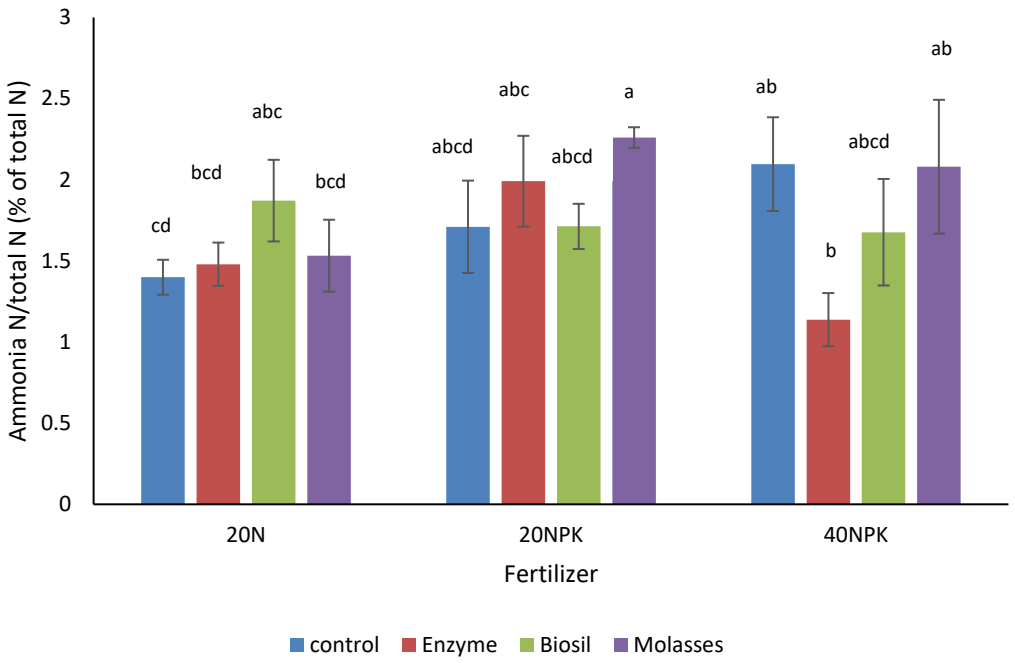

Figure 5.3. Effect of additive and fertilizer treatment on ammonia content of plantain silage.

The interaction between fertiliser and additive for lactic acid showed that the 20NPK fertiliser treatment generally resulted in the highest lactic acid except when the enzyme additive was used. (Figure 5.4.).

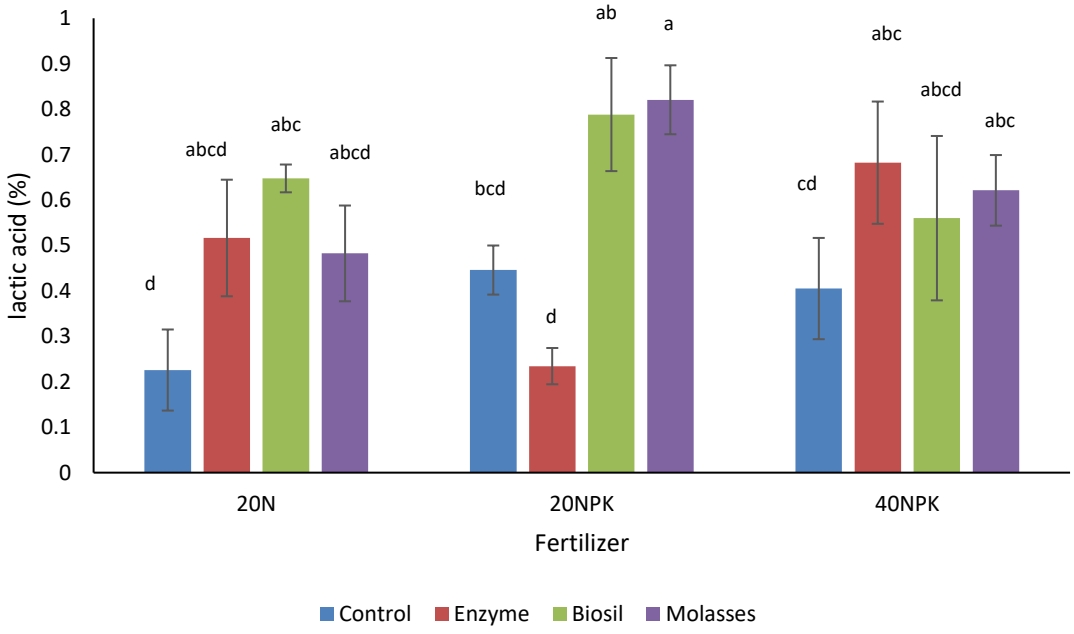

Figure 5.4. Effect of additive and fertilizer treatment on lactic acid percentage of plantain silage

5.3.5 Nutritive value of plantain silage

Nutritive characteristics for the mini silos are presented in table 5.4. There was no interaction between additive and fertilizer for any of the nutritive value variables of plantain silage. Fertilizer had little effect on the nutritive value of plantain silage other than to increase DM content in the 20N treatment. The use of additives had more impact on nutritive value than the fertiliser. Adding MOL reduced ADF and NDF ($P<0.05$) and tended to improve DM digestibility ($P<0.10$). The average crude protein was 11.6% and average digestibility was 50.7%. The organic matter content was similar in all treatments with an average value of 90.2%. Increasing N fertilizer reduced DM content.

Compared with the control, adding molasses reduced the NDF content and adding enzymes increased ADF content. The OMD and DOMD were similar among treatment, with an average of 48.6% and 43.9%, for OMD and DOMD, respectively. The metabolisable energy content, which was similar among treatments, was low with an average value of 7.02 MJ ME/kg DM.

Table 5.4. Effect of additive and fertiliser on nutritive value of plantain silage (%DM).

	DM (%)	OM (%)	CP (%)	ADF (%)	NDF (%)	DMD (%)	Ash (%)	OMD (5)	DOMD (%)	ME (MJ/kg DM)
Fertilizer										
20N	28.2 [a]	90.5	11.2	41.4	56.3	51.1	9.50	48.6	43.9	7.03
20NPK	25.2 [b]	90.2	11.8	42.4	56.9	50.5	9.82	48.3	43.6	6.97
40NPK	24.7 [b]	90.1	11.8	41.8	56.1	50.7	9.89	48.8	44.1	7.05
SEM	0.60	0.17	0.22	0.44	0.76	0.64	0.17	1.34	0.83	0.19
P Value	<0.001	0.24	0.08	0.31	0.59	0.84	0.24	0.95	0.91	0.91
Additives										
Control	25.6	90.43	11.4	41.9 [a]	57.3 [a]	50.0	9.57	47.4	42.9	6.87
Biosil	25.9	90.1	11.9	41.4 [a]	55.9 [ab]	51.4	9.93	48.8	43.0	7.04
Enzyme	25.9	90.1	11.7	43.4 [b]	57.5 [a]	49.7	9.92	47.5	43.0	6.87
Molasses	26.8	90.5	11.3	40.9 [a]	54.9 [b]	52.0	9.53	50.6	45.6	7.30
SEM	0.69	0.19	0.26	0.51	0.62	0.74	0.20	1.55	0.95	0.22
P value	0.65	0.32	0.44	0.01	0.02	0.09	0.32	0.17	0.17	0.17
Fertiliser x Additive	0.808	0.714	0.768	0.715	0.625	0.15	0.71	0.71	0.664	0.664

*Superscript of the different means in the same column differed significantly ($P<0.05$)
Where 20N=20 kg/ha of Nitrogen, 20NPK = nitrogen, phosphorus and potassium at 20:1:15 kg/ha;
40NPK = nitrogen, phosphorus and potassium at 40:1:15 kg/ha.
Dry Matter (DM), Organic Matter (OM), Crude protein (CP), Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD), Digestible Organic Matter in Dry matter (%) and Metabolisable Energy (MJ/kg DM).

5.4 Discussion

The hypothesis for the current study was that the use of fertilizer or additive would enhance the fermentation characteristics and nutritive value of plantain silage. The results of this study showed that firstly, the fermentation characteristics and nutritive value of plantain silage were relatively poor compared with recommended values and secondly, there was a limited effect of fertilizer or additive on fermentation characteristics. Fertiliser had a positive effect on yield (quantity), while only molasses added any benefit to fermentation characteristics.

Visual observation during the harvest period showed that the inclusion of P and K in fertilizer caused a growth response of other species e.g., white clover, ryegrass, and dicot weeds. This response from competition may have suppressed plantain yield. However, it can be noted here that shoot and seed head were similar at all fertiliser treatments. This made stem proportion similar for all treatments of silage.

5.4.1 Fermentation characteristics of plantain silage

The general observation of the plantain silages in the current study was similar to that observed in previous chapters. The plantain did not appear to be preserved well with respect to there being high pH and high BC, and a high percentage of mould as well. But on the positive side, the silage had a sweet smell, was low in NH_3-N (< 2%) and very low in butyric acid (<0.1%). The sweet aroma of plantain silage may come from the sugar alcohol sorbitol contained in plantain (Stewart, 1996). However, the role of sugar alcohol sorbitol in silage fermentation is unclear.

One of the most important attributes of good silage is low pH. Lactic acid bacteria produce lactic acid and acetic acid which drops pH then inhibit harmful bacteria growth. Both the low pH and the acids are beneficial in preserving the nutritive value of silage (Muck, 2010). In this study, pH of silages was more than 5, which is considerably higher than the target pH of 3.5 - 4.5 of silage (Howse *et al*. 1996). pH was lower at 20NPK when the enzyme was added. Whereas molasses reduced pH in the lowest level compared with other additives. Although the pH was still high at more than 5. The reason for this finding is molasses that comprised of sugar, acted as the energy source for LAB to grow (Gibbs *et al*. 1950),

The high pH in this study which was not affected by fertilizer and additives may because the silage was not preserved well. The high percentage of the stem in the herbage caused oxygen to remain in the silo. Once oxygen is present, yeast, mould and acetic acid bacteria begin to grow on silage, using fermentation products and residual sugar and producing CO_2, H_2O and heat (Muck, 2010). The presence of mould is not desirable because it indicates aerobic deterioration (Muck & Shinners, 2001). Considering there was visual evidence of mould observed inside the silos in this study (Table 5.3.), it is likely to have been high because of the high percentage of the stem (70% of DM) which made the silo's difficult to compress and pockets of oxygen remained near stem particles. We used the same type of plastic bag for mini silo in this study as with the previous chapters. It meant that they have the same oxygen permeability. There was evidently issues with oxygen in the silo due to mould growth but we believe this problem with oxygen ingress to the small silo was because the mature plantain with a high stem content was resilient so it was difficult to compress the bags to exclude oxygen completely from the silo. We closed the plastic by twisting the plastic bag. Although care was taken to eliminate oxygen from the plastic bags, we accept that there could have been oxygen ingress over time, as the thickness of the two plastic layers were unlikely to prevent oxygen from entering the silo completely (Borreani *et al.* 2007). Future studies should consider more suitable plastic materials and vacuum packaging for mini silo research. For the benefit of small holder farmers there is considerable value for the results of the small plastic bag to be applied to larger plastic bags. Using fertilisers to encourage leafy growth or molasses to encourage lactic acid bacteria help to reduce mould. Further, the high percentage of mould is less likely to occur in commercially produced silos (baleage or stack) as the raw material of silage is more likely to have greater compression by mechanical means than achieved by hand (Playne & McDonald, 1966).

Oxygen present in the silo contributed to the imperfect fermentation that has been shown by the low production of lactic acid (below 0.5%), whereas the target of lactic acid percentage in silage is at least 2% (Kung & Shaver, 2001). Water soluble carbohydrate content of plantain might be the other reason for the low production of lactic acid. Plantain composed of *L*-arabinose ,*D*-galactose, *D*-glucose, *D*-mannose, *L*-rhamnose , *D*-galacturonic acid, *D*-glucuronic acid and minor amounts of *L*-fucose. *D*-xylose (Brautigam & Franz, 1985) and sorbitol for about 42.1% of total WSC (Jiang *et al*. 2019), whereas sorbitol can not be fermented by LAB (Langston & Bouma, 1960).

Regarding buffering capacity, BC of plantain silage increased at a higher N fertilizer rate, accounted for more than 200 meq./100 g DM. In contrast, the good silage of ryegrass has BC of 112 meq/100 g DM (McDonald *et al*. 1991). This is consistent with the previous research (Keady & O'Kiely, 1996;

McDonald *et al*. 1991), who showed that an application of inorganic N fertilizer increased the BC of perennial ryegrass. The probable reason may because of the higher acetate production (Table 5.3.3) silage treated 40NPK as acetate could increase buffering capacity (Playne & McDonald 1966). Furthermore, the presence of phosphate will p;/ show some buffering action (Dunne, 1932). Wilkinson & Davis (2012) stated that BC may influence aerobic stability by affecting the rate of pH rise following the initiation of microbial activity on exposure of silage to air. Aerobic stability of silage is important because it relates to the safety and quality of the preserved forage upon exposure to air during storage and feeding (Li, Xu, Dong, Shi, & Zhang, 2016).

NH_3-N and butyric acid in this study was low (less than 0.5 %) while the requirement is less than 8-12% (Kung & Shaver, 2001). The higher ammonia was found at the higher nitrogen application, as urea is manufactured with anhydrous ammonia. The content of acetic acid and propionic acid also increased with the increase of the N application rate. Moderate acetic acid and propionic acid are beneficial for silage as they are good as antifungal acid (Woolford, 1984).

5.4.2 Nutritive value of plantain silage

In this study, the nutritive value of plantain was inferior with low protein content and lower ME values. This is because the crop stand had passed the bloom stage. When plants enter the reproductive phase, some nutrients are transferred to the generative organ, the nutrient content changes, and the forage quality falls (Tyrolová & Výborná, 2011). The fertiliser treatments had little effect on the nutrient content of fresh plantain that effect t the low nutrient content of the silage. It was expected with the higher rate of NPK that the nutritive value of the plant would increase because NPK comprised the vital mineral for the plant. The possible reason for the lack of response in this study may because only a low dosage of NPK was used. In a study with *Plantago arenaria*, (Hendawy, 2008) applied NPK of 100:100:50 increased its DM yield, carbohydrate, and mineral significantly. A similar finding was observed by Kołodziej (2006) for the utilization of NPK at a dosage of 60 : 40 : 80 increased phosphorus and potassium content of *Plantago lanceolata*. WSC of fresh or wilted plantain in this study around 7% (Table 5.2) that was enough for ensiling as the minimum WSC of herbage to have good silage is more than 2.5-3% (Haigh & Parker, 1985).

The low digestibility of plantain silages in this study was unexpected as it was only about 50% whereas the standard of DMD good pasture silage is more than 70%. Besides, their ME was not high (about 7 MJ ME/kg DM) and the standard of good pasture silage is more than 10 MJ ME/kg DM. Digestibility is a key factor for animal growth as reducing 1% of digestibility reduces intake by 50

g/day (Fitzgerald, 1987). The large drop in quality of this silage was probably due to the high mould content which indicated higher oxygen and onset of decay of the material. Further, low sugar substrate availability likely slowed LAB growth. The low overall quality of the silage is also because this silage had a higher proportion of stem that accounted for around 80%. High stem content was also the cause of the low CP content (less than 12%) whereas the standard of good silage is 17%. Again, a higher percentage of stem resulted in higher NDF content which has more than 55%. In this study, the use of additives decreased the NDF percent of plantain silage although it did not improve the digestibility of plantain silage. The NDF of this silage was higher compared to plantain silage observed by Raeside *et al*. (2012) who observed that the NDF of plantain silage was about 51%. However, the previous research has shown a lack of effect of inoculants (Meeske, 2005) or enzymes (Adesogan, Salawu, & Deaville, 2002) on silage quality. Lack of response to either of these additives is probably due to the high stem percentage of plantain silage which the enzymes are not well designed to digest in the plantain. Although molasses also failed to increase the digestibility, but it did reduce NDF percentage which might because it promoted bacterial activity. Comparing with the raw material of silage the digestibility decreased by 38% and ME decreased by 43%. High decrease in digestibility and ME might be triggered by the imperfect fermentation process, which makes silage become mouldy and mould used a lot of energy from silage that makes a significant loss of DM (Borreani, Tabacco, & Cavallarin, 2007).

Regarding crude protein, it was not undesirable the CP content of this silage was below 12% where the target of good silage is 17% (Howse *et al.* 1996) which is probably explained by the high stem content. DM content of silage was also not desired. The DM content of the silage was lower (<30% DM) compared to the DM target for good silage which is more than 30% (Howse *et al*. 1996). The low DM of silage in this study was because of the use of N fertilizer (Whitehead, 1995) and additives (Weinberg & Muck, 1996). The low DM% of silage is not beneficial for anaerobic stability. Low DM percentage of silage is not beneficial to inhibit clostridia growth as clostridia are susceptible to low water activity (Kleter, Lammers, & Vos, 1982).

5.5 Conclusion

These results demonstrate that 40NPK was better in fermentation characteristics in term of pH, total VFA and acetic acid that might be beneficial for aerobic stability. Whereas utilisation of molasses improved the fermentation characteristics in term of pH and BC. However, there was no interaction between fertilizer and additives for fermentation and nutritive value of plantain silage. Nutritive value of plantain silage at the late harvested could not be improved by fertiliser, but molasses improved the quality of plantain silage. However, the fermentation decreased feed value considerably to all treatments possibly due to O_2 ingress.

Chapter 6

In vivo digestibility in sheep

6.1 Introduction

As a consequence of variations in feed supply and feed demand in temperate pastoral systems, farmers need to preserve feed during a feed surplus, such as by ensiling, for animal feeding during a deficit. Currently, farmers utilize perennial ryegrass and white clover pastures for ensiling because this is the common pasture mix used in temperate farming. However, plantain (*Plantago lanceolata*) is becoming popular due to its mitigating potential for nitrogen losses (Box *et al*. 2016). The mechanism of plantain to reduce nitrate leaching maybe because of its secondary metabolites (aucubin and acteoside), or high water content, causing a diuresis effect and reduced nitrogen concentration in the urine, or both (Navarrete *et al*., 2016; O'Connell *et al*. 2016). As a fresh forage feed, plantain has many positive features compared to other forage feeds. For example, plantain is rich in minerals compared to ryegrass and white clover in terms of P, S, Ca, Na, Cu, Zn and Co (Harrington, Thatcher, & Kemp, 2006). Plantain has also been shown to support animal production when grown in mixtures (Bryant, Dalley, Gibbs, & Edwards, 2014) and as a pure sward (Box *et al*. 2016).

Less is known about the ensilability of plantain or how animals respond to plantain silage in terms of palatability or digestion. In the previous chapter (Chapter 3) it was shown that plantain, in the early stages of maturity, was of a better quality than plantain in the mature stage. Results in Chapter 5 showed that the addition of molasses resulted in better quality silage compared to other additives, particularly in late-harvested plantain. However, these previous trials were conducted at a laboratory scale (small silo) where surface area to mass ratio does not reflect typical conditions on commercial farms. Moreover, the response of animals to plantain silage has not been reported and it is important to identify whether plantain silage is acceptable to livestock.

Utilisation of silage to avoid waste is an important aspect of any feed in terms of economics, in addition to the quality, which can be a measure of its digestible energy and protein. *In vitro* assessment of feeds do not always reflect the animal's acceptability of the feed. Other than that, the palatability of plantain silage is still questionable as there is anecdotal evidence of aversion by animals to plantain at different times of the year. Further research is needed to understand whether palatability is improved or worsened on ensiling.

No less important is whether the diuresis effect, which has been linked with secondary compounds (Tamura & Nishibe, 2002) and high moisture content (O'Connell *et al*. 2017) still occurs if an animal is fed plantain silage. In the previous chapters (Chapters 3 and 4) we showed that the secondary metabolites (aucubin and catalpol) disappeared during ensiling and acteoside decreased markedly. Subsequently, urinary nitrogen losses from plantain silage may differ from those observed for fresh forages – or the mechanisms of altering urinary N loss may be different compared to fresh plantain. Therefore, the objective of this study was to compare the palatability, digestibility, nitrogen balance and nitrogen excretion patterns of plantain and conventional silage when offered to sheep. This study sought to test the following null hypotheses:

1. There is no difference in palatability of control and plantain silages
2. There are no differences in intake and digestibility of control and plantain silages
3. There is no difference in urinary N excretion of control and plantain silages

6.2 Materials and methods

6.2.1 Experimental site and design

The *in vivo* digestibility trial was conducted at the Johnstone Memorial Laboratory (JML) from 27 February until 22 March 2018 with approval from the Lincoln University Animal Ethics Committee (AEC 2018-03). The experimental design of the *in vivo* digestibility trial was a complete randomized block design with four silage treatments, and eight animal replicates across two runs (16 animals per run). The four treatments feed options were:

1. Ryegrass silage (4-week) silage, as a control
2. Plantain 4L (4-leaf appearance stage) silage
3. Plantain 6L (6-leaf appearance stage) silage
4. Plantain 6L + mol (molasses treated plantain at the 6-leaf appearance stage) silage

6.2.2 Silages

Harvest of the herbage for ensiling was carried out at LURDF between 23 November 2017 until 7 December 2017. Plantain (cv. Ceres Tonic) and perennial ryegrass (cv. Arrow) were used for ensiling and were sourced from the Lincoln University Research Dairy Farm (LURDF), Canterbury, New Zealand. Two paddocks, (farm ref: C1 and C2), which had been established in 2015 by sowing half of the paddock in plantain and half in perennial ryegrass, were selected for silage (Figure 6.1.).

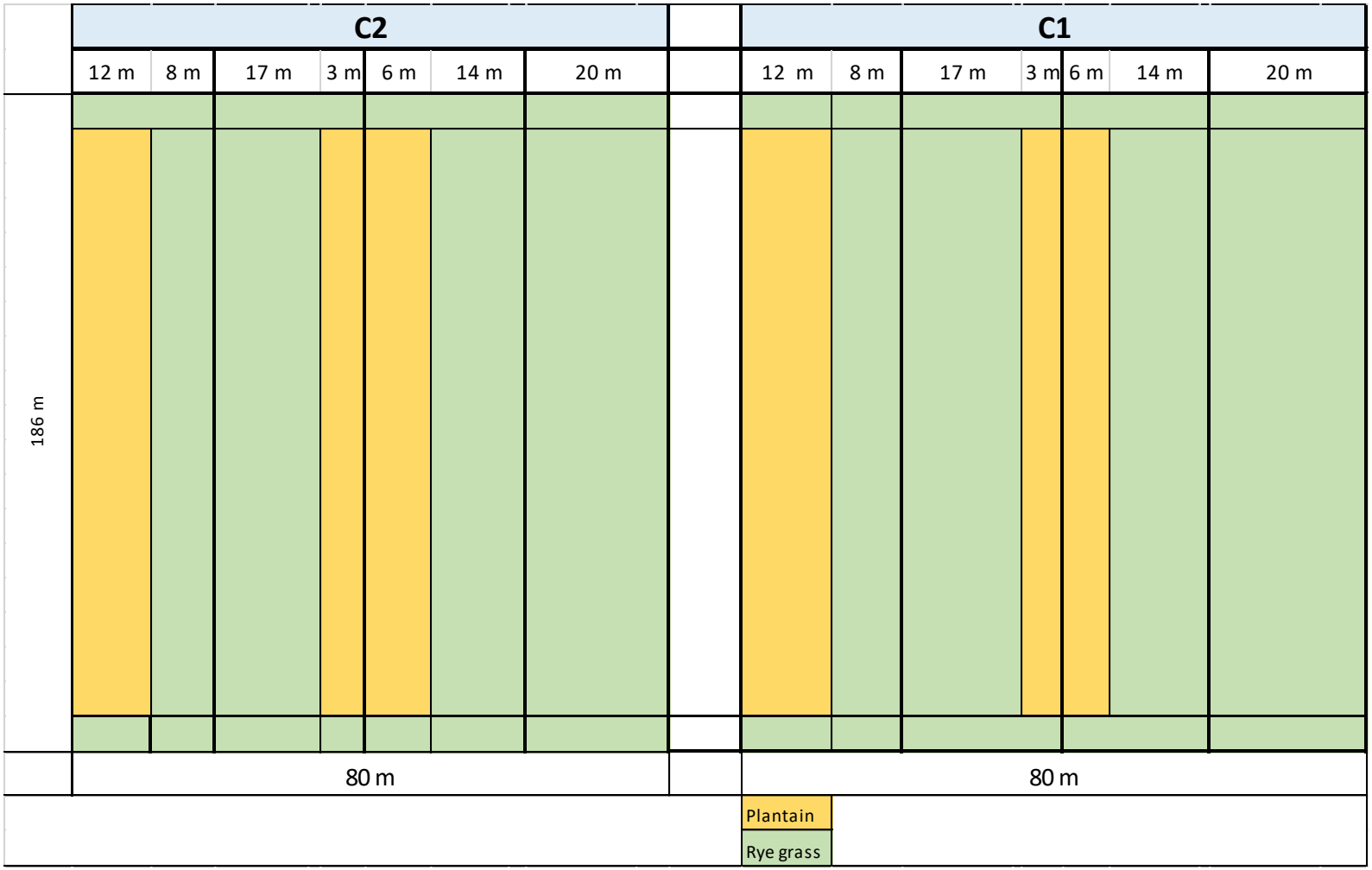

Figure 6.1. Map of plantain and ryegrass area (C1 and C2) (LUDRF, 2015).

The soil chemistry status, which was determined from soil sampling to 7.5 cm on 29 September 2017 from the area is shown in Table 6.1. The history of the area since establishment was rotational grazing by dairy cows, irrigation, and N fertiliser at a rate of approximately 150 kg N/ha. The area was prepared for silage by initially mowing on 22 October 2017, followed by fertilization with 25 kg N/ha.

Table 6.1. Soil fertility status of plantain and ryegrass areas in 2017 (LURDF, 2017)

Parameter	Level found	Medium range
pH	6.4	5.8-6.2
Olsen phosphorus (mg/L)	23	20 – 30
Potassium (me/100 g)	1.08	0.4 - 0.6
Calcium (me/100 g)	8.6	4.0 – 10.00
Magnesium (me/100 g)	1.09	1.00 – 1.6
Sodium (me/100 g)	0.16	0.2 - 0.5
CEC (me/100 g)	14	12 – 25
Total base saturation (%)	76	50 – 85
Volume weight (g/mL)	0.92	0.6 – 1.00
Sulphate sulphur (mg/kg)	3	10 – 12

When plantain pastures reached target development (4-leaf and 6-leaf appearance), agronomic data was collected for the different pastures. Herbage yield was determined by harvesting replicated (n=6) quadrats (0.2 m^2) to ground level with an electric handpiece. Harvested samples were then oven dried at 60°C for 48 hours to a constant weight. Botanical composition was determined from approximately 25 g sample within each quadrat. These samples were then oven dried at 60°C for 48 hours. After recording the dry weight of the sub-sample, the value was then combined to the bulk quadrat weight for yield determination.

Plantain reached 4-leaf stage on 23 November and 6-leaf stage on 6 December. Assuming a base temperature of 5°C the growing degree days (GDD) to reach 4L stage (four-leaf appearance) was 309 and 497 GDD to reach 6L stage. Allocated areas were then mown to 6 cm residual height in the afternoon (between 13.00-14.00 hours). At the same time as the plantain 4-leaf stage was mown, the ryegrass pasture in the same paddock was also mown. Assuming a base temperature of 0°C, the growing degree day for ryegrass was 474 on 23 November.

To determine the DM percentage of fresh plantain and ryegrass, a bulk sample of 50 g of fresh samples after mown were taken then they were subjected to oven drying at 60^{o}C for 48 hours. Another 50 g of fresh sample was taken and freeze dried, ground and then analysed for nutrient content. The nutrient contents (ADF, NDF, CP, WSC, OM, and DOMD) of herbage were measured by using NIRS (NIRS 5000, Foss, Maryland, USA). Calibration equations for NIRS were derived from perennial ryegrass, clover and plantain herbage. ME was calculated using the equation stated by CSIRO (2007):

ME (MJ/kg DM) = 0.16 x DOMD

Secondary metabolite compounds (catalpol, aucubin and acteoside) analysed for plantain only using high performance liquid chromatography (HPLC) by Lincoln University analytical laboratory following procedures of Tamura and Nishibe (2002). The sample used for the secondary metabolites was a bulk sample fresh and silage plantain, so the total number of samples was five. Two fresh samples of plantain 4L and 6L. The other samples were from plantain silage of 4L, 6L and 6L+mol which were taken from baleage before trial.

Both plantain and ryegrass pastures were wilted for 24 hours until they passed the squeeze test. The wilted plantain and ryegrass were baled using a 150 hp Case tractor (with a McHale Fusion 3 Plus baler wrapper combination). The McHale Fusion generated round bales with four layers 25 μ Baletite® plastic and four layers of 19 μ Silotite® (total 180 μm of plastic layer). Dry matter percentage of forage prior to ensiling was confirmed by oven drying a 50 g (of bulk samples) sub-sample for 48 hours at 60^{o}C. Another 50 g of samples were also taken for freeze drying, ground to a 1 mm sieve and analysed for nutrient content by using NIRS.

At 6-leaf stage (December) half of the area was immediately baled as described above while the other half was subjected to molasses-treatment. Molasses was added by pouring the molasses evenly on the surface area of the wilted plantain before being baled by using a 5L bucket. This required 11 times of pouring covering all wilted plantain in the determined area. The dosage of molasses was 20 L/ton of fresh plantain (Castle & Watson, 1985). The total amount of molasses used was 85 L for 4.3 tons of fresh plantain from 2,232 m2 of harvested plantain area. The molasses-treated plantain was then baled as described above. Each bale (200 kg DM) for ryegrass, plantain 4L, plantain 6L silages and 2 bales for plantain 6L+ mol silage were then stored outdoors for three months prior to use in the *in vivo* trial.

a).

b).

Plate 6.4. Plantain 4L baleage (a) and ryegrass silage (b)

c).

d).

Plate 6.5. Plantain 6L baleage (c), and plantain 6L+mol baleage (d)

6.2.3 Animals, feeding and measurement

Thirty-two male lambs between 6 and 12 months of age were used for this study. Prior to the study the lambs were grazed on perennial ryegrass and white clover pasture. On 27 February 2017, the lambs were weighed and screened for suitability for a metabolic stall study by selecting animals that

adapted well to being in the crates for 24 hours (i.e. accepted the feed offered and did not try to jump out of the crates). After weighing, the lambs were blocked into groups based on live weight (35.99 ± 2.11 kg LW) and then allocated randomly to a treatment. In vivo digestibility was determined on four lambs per treatment per run. Each run lasted 10 days including a five day palatability study and five day digestibility measurement period. At the end of each 10-day run the lambs were reweighed and released to pasture and three days later a new batch of 16 lambs (four lambs per treatment) was weighed and selected.

Animals were fed once daily in the morning between 09.00 and 10.00 h. For the study a single bale for each treatment was used for both runs during the study, with the exception of 6L+mol which became very mouldy during the second run, so a second bale was opened in the day 7 of the second run. Mould growth was evident at the face of all bales so each day the surface of the bale of silage was removed and discarded. Clean silage was then pulled from the bale, weighed and offered to lambs. Fresh, clean water was available ad-lib.

The silage allocation was ad lib by estimating the ME requirements of the lambs for maintenance and growth (Table 6.2) and increasing allocation by 10%. The ME requirement of the lamb followed the equation stated by CSIRO (2007).

$$ME_m\ (MJ/d) = K \times S \times (0.28W^{0.75} \times \exp(-0.03A))/km + 0.1ME_p \times 1.05$$

Where: K = 1.0 for sheep

S = 1.15 for entire males

W = live weight (kg)

ME_p = the amount of dietary ME (MJ) being used directly for production

A = Age in year, with a maximum value of 6

Km = net efficiency of use of ME for maintenance

1.05 = 5% safety factor

To determine the ME value of the diets, a subsample (50 g) of silages were taken randomly from the bales, then oven dried for 48 hours at 60^{o}C. The ME values of silages were measured by NIRS by multiplying 0.16 of the silages DOMD.

Table 6.2. Example of estimation of silage allocation to the animals each day.

Silage	ME maintenance /day (MJ/head/day)	ME growth /day (MJ/head/day)	Total ME req (MJ/head/day)	Diet ME (MJ/kg DM)	Minimum allocation (kgDM/day)	Feed DM (%)	Fresh offered + 10% (kg)
Ryegrass	7.3	8	16.1	11.4	1.4	47.4	3.3
Plantain 4L	7.3	8	16.1	10.2	1.6	36.1	4.9
Plantain 6L	7.3	8	16.1	9.0	1.8	44.2	4.5
Plantain 6L+MOL	7.3	8	16.1	9.4	1.7	39.0	4.8

In this experiment, the sheep were fed *ad libitum* to order to ensure if there were differences in intake because of the difference of silage type and maturity. To ensure the *ad lib* status, feed refusal was maintained at 10% of the allocation. If the feed refusal was more than 10% of the total, the amount of feed offered was decreased. The feed offered and refused was recorded daily using electronic scales. Dry matter intakes (DMI) were estimated by determining the difference between the herbage mass offered and herbage remaining. Feed intake was determined over days one to ten by weighing refused feed and determining the proportion of offered feed consumed.

Fresh water was provided twice daily, at 10.00 and 15.00 h, and the volumes of water drunk were measured. Water intake in this trial was measured by combining the water consumed and the water contained in the feed.

Urine and faecal excreta were measured at day 6 to day 10 from collection trays and buckets under the metabolism crates. A separator was used to trap the faeces and this allowed the urine to pass through into a collecting bucket. Urine was acidified by placing 250 mL of a 5% sulphuric acid solution in the collection bucket to prevent volatilisation of N. Urine volumes were measured in volumetric flasks. Approximately 70 mL from each sample was stored frozen for further analysis. Samples of urine and feces from day 6-10 were bulked for each animal and were analysed for N content by using elementor analyser Vario Max CN analyser which used catalytic tube combustion under oxygen supply and high temperature.

DM intake was determined from the average value from day six to day ten. *In vivo* digestibility was measured by deducting the amount of DM consumed from the amount of DM excreted in faeces over the amount of DM consumed in the last five days (day 6 -10).

Analysis of the *in vivo* DM Digestibility (% DMD , % Organic Matter Digestibility (OMD), % Digestible Organic matter in Dry Matter (DOMD), Metabolisable Energy (ME)(MJ/kg DM) followed the methods described by (CSIRO, 2007).

$$\textit{In vivo}\ \text{DMD}\ (\%) = \frac{\text{Amount of DM consumed} - \text{amount of DM excreted in faeces}}{\text{Amount of DM consumed}} \times 100\%$$

$$\textit{In vivo}\ \text{OMD}\ (\%) = \frac{\text{Amount of OM consumed} - \text{OM excreted in faeces}}{\text{Amount of OM consumed}} \times 100\%$$

In vivo DOMD (%) = 100 % x (Feed OM – Faeces OM)/Feed DM

ME intake (MJ/kg DM) = (0.16 x DOMD) x total DM intake

Silage characteristics were measured three times during each run, at days 1, 5 and 10. The total samples of silage characteristics was 24 comprised of 4 treatment of silage, 2 runs and 3 dates as replication. Silage characteristics include pH, BC, total VFA, lactic acid, acetic acid, propionic acid and total NH_3-N per total N based on fresh silage. In brief, pH and BC were measured following the method described by Playne and McDonald (1966). Total VFA, acetic acid and propionic acid were measured by gas chromatography (GC 2010, Shimadzu, Japan). Other silage characteristics; namely, lactic acid, total NH_3-N per total N, were measured using a Randox analyser (Rx Daytona,UK).

For measuring DM%, a 50 g of samples from the feed offered were taken each day before feeding the animal. The total samples were 32 which consisted 4 treatments, 4 replications (animals were as replications) and 2 runs. The feed refused measurements were also taken approximately 50 g every day. The total was 40 samples, which consisted 4 treatments, 5 dates as replications and 2 runs.

Feed quality and DM%: The diets were also determined for ADF, NDF, ash, OM on a DM basis by using NIRS with dry silage (non-starch) calibration**.** The total samples of calibration was 129. The total samples was 32, which consisted of 4 treatments, 4 replicates and 2 runs. The quality and botanical composition of the diets consumed were determined from a selection of the feed offered and refused.

Samples for botanical composition were taken every day (day 6 until day 10), then pooled. So, the samples of botanical composition consisted 4 treatments, 5 replications, and 2 runs. Date sampling was a replication. The total sample was 40.

Plate 6.6. *In vivo* digestibility trial

6.2.4 Nitrogen balance

The nitrogen balance, retained nitrogen and nitrogen excretion patterns in this trial were measured based on the equation based on AFRC (1993), below:

Nitrogen balance (g/head/day) = amount of nitrogen consumed – nitrogen excreted in faces and urine– nitrogen retained in liveweight gain

$$\text{Nitrogen retained in liveweight gain (g/head/day)} = \frac{\Delta W\,(160.4 - 1.22W + 0.0105\,W^2)}{6.38}$$

Where ΔW = liveweight gain

W = Initial weight

$$\text{Nitrogen excretion pattern} = \frac{\%\ \text{Nitrogen intake in urine}}{\%\ \text{Nitrogen intake in faeces}}$$

$$\%\ \text{Nitrogen intake in urine} = \frac{\text{Total nitrogen excretion in urine}}{\text{Nitrogen intake}} \times 100\%$$

$$\%\ \text{Nitrogen intake in faeces} = \frac{\text{Total nitrogen excretion in faeces}}{\text{Nitrogen intake}} \times 100\%$$

6.2.5 Statistical analysis

Fermentation characteristics of herbage silage; botanical composition of feed offered and refusal were analysed using ANOVA Genstat 19.1 (VSN international Ltd, 2018) with silage as a fixed term and run as a random term. Chemical characteristics of herbage silage, digestibility and N balance variables were compared for each silage using silage as fixed terms and run as a block. This used ANOVA Genstat 19.1. (VSN International Ltd, 2018) for data analyses.

6.3 Results

6.3.1 Pre-Ensiling

The DM production of ryegrass and plantain at the early stage of maturity showed no difference (Table 6.3). Higher production was found at the late stage of maturity (6-leaf of plantain). The percentage of leaf was highest at ryegrass compared to that of plantain 4L and lowest for plantain 6L. The leaf percentage of plantain 4L was accounted for three times higher than that of plantain 6L. Seedhead of ryegrass was less than 1%. In contrast, seed head of plantain 6L was more than 50% of

its DM. Plantain 4L had a moderate percentage of seed head with less than one-third of plantain seed head. There was less than 10% clover in all cut material prior to ensiling. Weed was three times greater at plantain 6L compared to ryegrass and plantain 4L. However, dead material was greater at ryegrass prior to ensiling.

The wilting process resulted in a greater loss of moisture in the ryegrass and the least moisture loss in the plantain 4L. The ADF of ryegrass at the early regrowth stage was lower than that of plantain at the late stage of maturity (Table 6.3). In plantain 6L, the ADF increased by 4% in the wilted plantain. At plantain 6L, NDF was also higher in the wilted plantain with a difference of 8.6%. In terms of crude protein, the CP in wilted material was higher compared to the fresh plant, except for plantain 4L. At the early stage of maturity, crude protein was greater compared to the late stage of maturity. The WSC of herbage in this study tended to increase after wilting. WSC plays an essential role for ensiling due to its role in producing lactic acid that prevents nutritive value of silage. WSC ryegrass made up more than 20% of DM. WSC of fresh and wilted plantain 4L accounted for more than 10%, and barely 10% for fresh and wilted plantain 6L.

The organic matter (OM) among treatments was not different neither was the OM between the fresh and wilted plantain. Metabolisable energy was higher in ryegrass, followed by plantain 4L and plantain 6L.Regarding the secondary metabolites, there was not a difference between the secondary metabolites of plantain harvested at the early stage of maturity and the late stage of maturity in fresh plantain (Table 6.3). Among the secondary metabolite content in plantain (at all stages of maturity), the highest proportion was acteoside.

Table 6.3. Physical and Chemical characteristics of plantain 4-leaf appearance (4L), 6-leaf appearance and ryegrass prior to ensiling (% DM).

	Ryegrass	Plantain 4L	Plantain 6L
		Fresh	
Herbage yield (kg DM/ha)	4,004	4,063	4,988
Leaf (%DM)	67.9	44.8	14.7
Seed head (% DM)	0.26	20.2	54.1
Plantain (% DM)	2.27	67.7	71.0
Ryegrass (% DM)	72.5	9.97	3.34
Clover (% DM)	5.49	8.23	2.82
Weed (%DM)	2.38	2.76	8.90
Dead material (%DM)	22.8	15.3	14.2
Dry Matter (%)	20.6	22.7	26.2
Organic Matter (%)	90.4	88.9	90.4
Dry matter digestibility (%)	79.9	74.5	69.5
Acid Detergent Fibre (%)	22.9	23.0	27.3
Neutral Detergent Fibre (%)	37.6	34.8	42.1
Crude Protein (%)	14.9	14.2	9.30
Water Soluble Carbohydrate (%)	27.2	10.2	7.50
Metabolisable Energy (MJ /Kg DM)	12.7	11.7	10.5
Aucubin (mg/g)	-	0.23	0.21
Catalpol (mg/g)	-	1.82	1.99
Acteoside (mg/g)	-	10.6	10.7
		Wilted	
Dry Matter (%)	54.2	36.9	46.1
Organic Matter (%)	90.1	88.1	92.4
Dry matter digestibility (%)	78.6	76.3	68.5
Acid Detergent Fibre (%)	21.6	22.8	29.7
Neutral Detergent Fibre (%)	38.6	33.4	46.1
Crude Protein (%)	16.5	14.4	12.5
Water Soluble Carbohydrate (%)	24.6	11.0	9.40
Metabolisable Energy (MJ/kg DM)	12.5	11.8	11.0

6.3.2 Post harvest silage

The fermentation characteristics of herbage silages showed differences among the treatments (Table 6.4). The pH of silages in this study was high where its pH was more than 5. The BC presented showed evidence that ryegrass and plantain 4L silage were higher compared to those of plantain 6L and plantain 6L + mol. The total VFA in this study were very low where the highest was only almost 1% and it was in the plantain 4L. Other silage diets were below 0.5%. Not only percentage of lactic acid that was greater at plantain 4L silages but also the percentage of acetic acid present in this silage was the greatest ($P<0.05$). However, the proportion of propionic acid, butyric acid and NH_3-N per total N did not differ among silage diets. It can be seen in Table 6.4 that there was no propionic acid produced and only a tiny amount of butyric acid content in all silage diets. NH3-N per total N production in this study was low in all silages and the highest was at the plantain 4L silage.

Table 6.4. Fermentation characteristics of ryegrass and plantain 4-leaf appearance (plantain 4L), 6-leaf appearance (plantain 6L), 6-leaf appearance + molasses (plantain 6L+mol) silages

Variable	Ryegrass	Plantain 4L	Plantain 6L	Plantain 6L+mol	SEM	P value
pH	5.62	5.42	6.21	5.76	0.29	0.293
BC (meq/100g DM)	320 ^{a}	341 ^{a}	169 ^{b}	214 ^{ab}	32.5	0.003
Total VFA (%)	0.32^{b}	0.91^{a}	0.31^{b}	0.40^{b}	0.11	0.045
Lactic acid (%)	0.06 ^{b}	0.51 ^{a}	0.18 ^{ab}	0.19 ^{ab}	0.09	0.044
Acetic acid (%)	0.22	0.36	0.15	0.19	0.05	0.169
Propionic acid (%)	0	0.002	0	0.002	0.001	0.56
Butyric acid (%)	0.01	0.02	0.001	0.01	0.01	0.423
NH_3-N (% of total N)	1.07 ^{c}	3.88 ^{a}	1.75 ^{bc}	2.86 ^{ab}	0.19	0.012

*Superscripts in the same row differed significantly ($P<0.05$)
S X R = Silage x Run
BC = Buffering capacity; VFA = volatile fatty acid, NH_3-N = Nitrogen in ammonia

The indicative nutritive values of ryegrass and plantain silage are presented in Table 6.5. It can be seen that there were large differences in nutritive value between silage at the early stage of maturity and at the late stage of maturity. Generally, the quality of the silage declined with increasing age of plant as shown by higher fibre content in the late stage of maturity (plantain 6L and plantain 6L + mol).

Treatment effects revealed the lowest ADF, NDF and highest ME in ryegrass silage. However, plantain 4L silage was superior in terms of ADF, NDF and CP content compared to other plantain silages. ME content was unaffected by regrowth stage and additive at plantain silages. Compared with pre-ensiled forage the concentration of secondary compounds had nearly disappeared with only trace amounts of acteoside, and catalpol remaining.

Table 6.5. Chemical characteristics of ryegrass, plantain 4-leaf appearance (plantain 4L), plantain 6-leaf appearance (plantain 6L), plantain 6-leaf appearance treated molasses (plantain 6L + mol) silage (% DM).

	Ryegrass	Plantain 4L	Plantain 6L	Plantain 6L + mol	SEM	P value
DM (%)	51.8 ^{b}	36.4 ^{c}	57.1 ^{a}	50.2 ^{b}	1.20	<0.001
ADF (%)	24.3 ^{d}	32.2 ^{c}	38.1 ^{a}	35.0 ^{b}	0.47	<0.001
NDF (%)	41.2 ^{c}	45.4 ^{b}	57.6 ^{a}	56.2 ^{a}	0.78	<0.001
CP (%)	19.0 ^{a}	15.2 ^{b}	11.4 ^{d}	13.1 ^{c}	0.41	<0.001
WSC (%)	14.0 ^{a}	4.35 ^{c}	6.82 ^{b}	7.18 ^{b}	0.59	<0.001
OM (%)	93.2 ^{a}	90.1 ^{b}	92.1 ^{ab}	91.7 ^{ab}	0.53	0.003
DMD (%)	72.6 ^{a}	61.2 ^{b}	54.2 ^{c}	56.8 ^{bc}	1.56	<0.001
ME (MJ/kgDM)	11.4 ^{a}	9.71 ^{b}	8.87 ^{b}	9.5 ^{b}	0.41	0.001
Aucubin (mg/g)	-	0	0	0	-	
Catalpol (mg/g)	-	0.36	0	0	-	
Acteoside (mg/g)	-	0.69	0.16	0.31	-	

Plantain 4L = four leaves appearance in plantain
Plantain 6L = six leaves appearance in plantain
Plantain 6L + mol = six leaves appearance in plantain treated molasses
*The different superscript in the same row differed significantly (P<0.05)

6.3.3 Animal response to silage

The effect of silage type on palatability (as determined by feed intake from day 1 – 10) is shown in Figure 6.2. Sheep took five days to acclimatise. All animals took time to adapt to their treatments with most sheep consuming relatively consistent quantities by day 6. Sheep fed ryegrass and plantain 4L silage were relatively more consistent in intake.

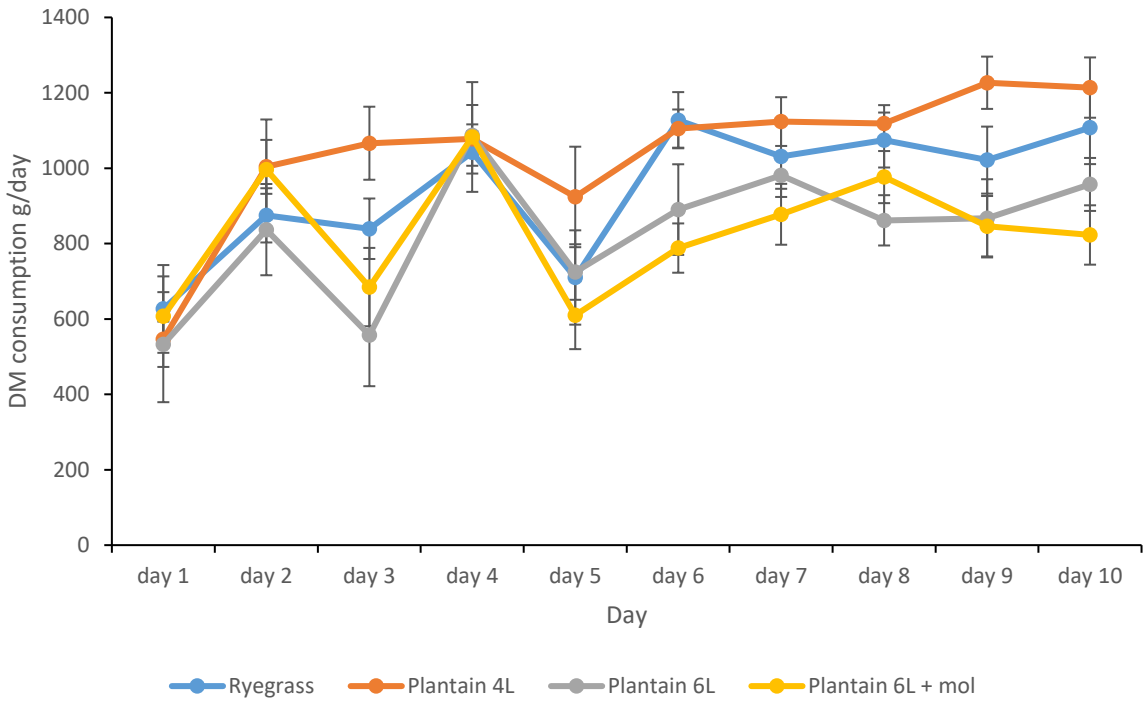

Figure 6.2. Intake of ryegrass, plantain 4-leaf appearance (PL 4L), plantain 6-leaf appearance (PL6), plantain 6-leaf appearance treated molasses (PL 6L+mol) silages from day 1-10.

Ryegrass silage was dominated by leaf in feed offered (±90%) while the plantain silages consisted mainly of stem (Table 6.6.). Lambs actively sought to consume more leaf and clover, leaving more stem in the refusals than other plant components.

Table 6.6. Botanical composition of feed offered and refusal of ryegrass, plantain 4-leaf appearance (PL 4L), plantain 6-leaf appearance (PL6), plantain 6-leaf appearance treated molasses (PL 6L+mol) silages.

Variable	Ryegrass	Plantain 4L	Plantain 6L	Plantain 6L+mol	SEM	P value
			Feed offered			
Ryegrass leaf	88.2 ^{a}	11.8 ^{b}	1.70 ^{c}	12.8 ^{b}	0.30	<0.001
Ryegrass stem	3.40 ^{b}	10.5 ^{b}	18.3 ^{a}	37.1 ^{a}	0.39	<0.001
Plantain leaf	2.4 ^{c}	21.5 ^{a}	8.30 ^{b}	5.96 ^{b}	2.16	<0.001
Plantain stem	1.50 ^{b}	38.7 ^{a}	56.1 ^{a}	35.9 ^{a}	0.18	<0.001
Clover	3.70 ^{b}	9.59 ^{a}	3.2 ^{b}	2.06 ^{b}	0.19	<0.001
Weed	0.77 ^{c}	7.97 ^{ab}	12.4 ^{a}	6.22 ^{b}	0.18	<0.001
			Feed refusal			
Ryegrass leaf	74.7 ^{a}	4.5 ^{b}	4.5 ^{b}	1.7 ^{b}	0.46	<0.001
Ryegrass stem	17.4 c	7.10 ^{bc}	15.2 ^{ab}	35.6 ^{a}	0.43	<0.001
Plantain leaf	0.85 b	8.40 ^{a}	4.60 a	4.92 b	0.25	<0.001
Plantain stem	2.38 b	67.8 a	61.7 a	51.7 a	0.30	<0.001
Clover	3.40 ^{a}	5.10 ^{a}	2.75 ^{ab}	0.96 ^{b}	0.2	0.002
Weed	1.27 ^{c}	7.07 ^{ab}	11.3 ^{a}	5.16 ^{b}	0.21	<0.001

*The different superscript in the same row differed significantly (P<0.05)

The dry matter digestibility of all plantain silages were less than 70%, with the ryegrass silage being more digestible than either of the plantain silages (Table 6.7). There was large variability in digestibility between animals, which meant it was not possible to detect a significant difference in digestibility between ryegrass and plantain 4L, which were numerically different by 10 DMD% units. However, variation in OM content did reveal significant differences in DOMD between the control and plantain 4L.

High fibre and low digestibility likely limited apparent intake of plantain 6L as lambs on those diets consumed only two-thirds of what they were allocated. As a result, the ME intake of plantain 4L silage was higher compared with the other plantain silages, which was, in turn, lower than that of ryegrass silage.

While there was no difference in the absolute values for live weight at the start and at the end of the study, the weight gain of sheep was affected by the type of silage. Weight gain of sheep fed ryegrass silage was more than three times higher of weight gain of sheep fed plantain 4L silage while feeding plantain 6L and plantain 6L + mol resulted in weight loss.

Table 6.7. Dry matter and metabolisable energy intake and *in vivo* digestibility of ryegrass, plantain 4-leaf appearance (PL 4L), plantain 6-leaf appearance (PL6), plantain 6-leaf appearance treated molasses (PL 6L+mol) silages offered to sheep.

Variable	Ryegrass	Plantain 4L	Plantain 6L	Plantain 6L+mol	SEM	P value
DM offered (g/animal/day)	1,592 ^{a}	1,514 ^{a}	1,526 ^{a}	1,381 ^{b}	30.4	<0.001
DM refusal (g/animal/day)	520 ^{ab}	357 ^{b}	615 ^{a}	530 ^{ab}	45.7	0.004
DM intake (g DM/animal/day)	1,072 ^{ab}	1,177 ^{a}	926 ^{bc}	848 ^{c}	62.5	0.004
DM faeces (g DM/animal/day)	279 ^{c}	404 ^{a}	357 ^{ab}	307 ^{bc}	25.3	0.007
In vivo DMD (%)	76.5 ^{a}	66.5 ^{ab}	59.1 ^{b}	64.6 ^{b}	3.53	0.014
In vivo OMD (%)	76.5 ^{a}	67.3 ^{b}	60.2 ^{b}	64.7 ^{b}	2.66	0.002
In vivo DOMD (%)	71.3 ^{a}	60.7 ^{b}	55.4 ^{b}	59.4 ^{b}	2.53	0.001
ME intake (MJ ME/day)	12.0 ^{a}	9.56 ^{b}	7.28 ^{c}	6.91 ^{c}	0.61	<0.001
Initial weight (kg)	36.2	36.2	35.9	35.8	1.38	0.995
Final weight (kg)	38.2	36.63	35.6	35.3	1.31	0.39
Weight gain (kg)	2.03 ^{a}	0.61 ^{ab}	(0.33) ^{b}	(0.5)^{b}	0.54	0.01

DM = dry Matter; ( ) = a weight loss of animal.
The different superscript in the same row significantly differed (P<0.05)

The positive relationship between digestibility of silage and weight gain of animals (Figure 6.3) demonstrated that high digestibility resulted in high weight gain whereas poor digestibility (below 63%) led to a weight loss of the animal.

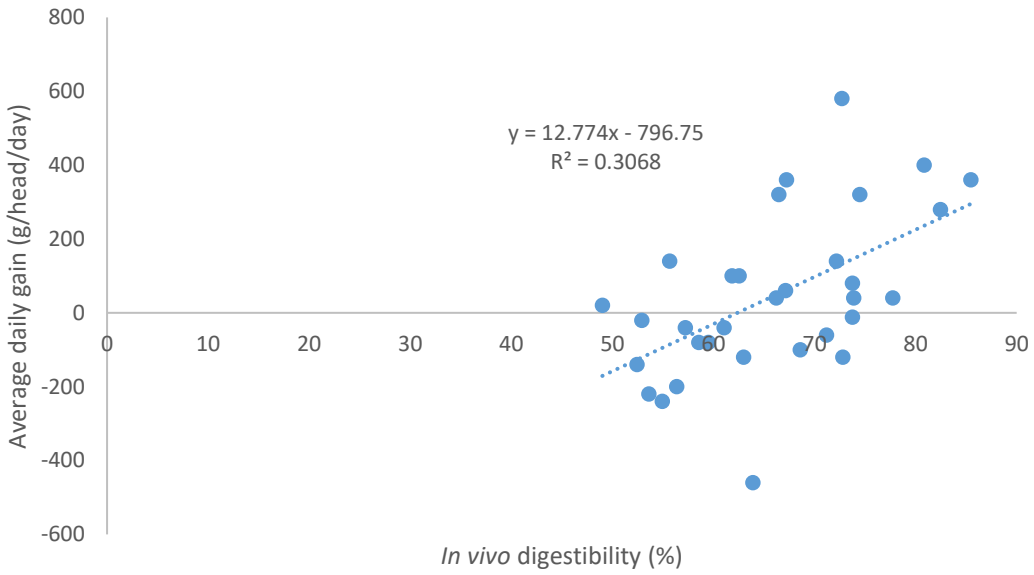

Figure 6.3. The relationship between *in vivo* digestibility and weight gain of animal

There was no evidence of a diuresis effect from feeding plantain silage. The average urine volume was 1.48 litres and treatment did not affect the total urine excretion. However, total water consumed was highest for lambs with the highest DM intake (i.e. ryegrass silage and plantain 4L silage). Differences in apparent N intake corresponded with differences in the CP% and maturity of the silages with sheep fed ryegrass silage consuming the most N, followed by plantain 4L (Table 6.8). In lambs fed plantain, relatively more of the consumed N was excreted in faeces. N excreted in urine per animal per day was highest in sheep fed ryegrass silage. The lowest urinary N excretion was recorded in sheep fed plantain 6L, where the value was almost a half from sheep fed plantain 4L. The total nitrogen retained was higher in sheep fed ryegrass silage followed by plantain 4L silage. Neither plantain 6L nor plantain 6L +molasses gave positive nitrogen retained to the sheep.

A large proportion of the apparent N intake could not be explained by N measured in urine dung or live weight with lambs on the ryegrass silage having the greatest variation in N balance.

Table 6.8. Nitrogen balance, nitrogen retention and nitrogen excretion pattern (urinary N/faecal N)

Variable	Ryegrass	Plantain 4L	Plantain 6L	Plantain 6L+mol	SEM	P value
N intake (g/head/day)	32.5 [a]	27.9 [b]	18.1 [c]	16.7 [c]	0.99	<0.001
Water intake (L/head/day)	4.26 [ab]	4.73 [a]	3.23 [b]	3.28 [b]	2.56	<0.001
Urine volume (L/head/day)	1.72	1.69	1.33	1.17	0.19	0.134
Urine N concentration (g/L)	5.27 [a]	3.98 [ab]	3.40 [b]	4.46 [ab]	0.43	0.03
Faecal N concentration (g N/kg DM Faeces)	37.7 [a]	35.8 [a]	28.9 [b]	28.9 [b]	0.72	<0.001
N excreted in urine (g/head/day)	8.61 [a]	6.33 [ab]	3.99 [b]	5.52 [ab]	0.67	<0.001
N excreted in faeces (g/head/day)	8.54 [b]	13.5 [a]	9.44 [b]	8.00 [b]	0.74	<0.001
N retained in ADG (g/head/day)	4.13 [a]	0.99 [ab]	(0.64) [b]	(1.03) [b]	1.14	0.014
N balance (g/head/day)	11.3 [a]	7.15 [ab]	3.90 [b]	5.58 [b]	1.55	0.016
% of N intake in dung	26.4 [b]	49.2 [a]	56.4 [a]	46.5 [a]	2.80	0.049
% of N intake in urine	26.4	22.8	24.3	30.2	3.01	0.361
Urinary N / faecal N	1.10 [a]	0.53 [c]	0.46 [c]	0.79 [b]	0.09	<0.001

Plantain 4L = plantain 4- leaf appearance, Plantain 6L = plantain 6-leaf appearance ;
Plantain 6L + mol = plantain 6-leaf appearance treated molasses
* The different superscripts in the same row show significant different (P<0.005)
( ) = negative

For nitrogen excretion pattern, there was a positive relationship between nitrogen excretion pattern and total N consumed (P =0.020). Higher nitrogen consumed resulted in higher nitrogen excretion pattern. (Figure 6.4). Though there was considerable variation as reflected by low R^2 of 0.17.

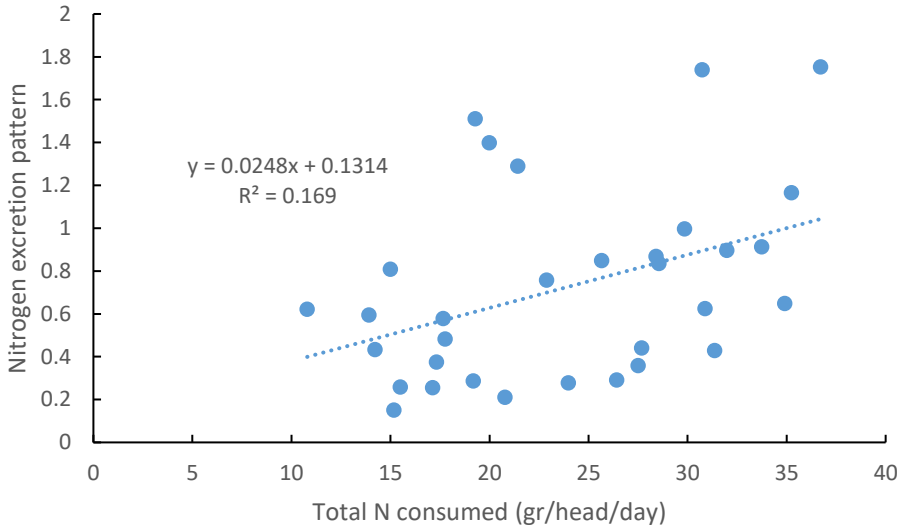

Figure 6.4.The relationship between Total N consumed (g/head/day) and nitrogen excretion pattern

6.4 Discussion

The objective of this study was to compare silage quality (at a commercial size) for conventional ryegrass with plantain silages at different maturity on intake, digestibility, and nitrogen utilisation by lambs. It was hypothesised that at the same maturity, ryegrass and plantain silage would have similar digestibility but better N utilisation from plantain. Further, it was hypothesised that adding molasses to very mature plantain silage would improve acceptability and digestibility. All these hypotheses, we were unable to accept, as explained below.

6.4.1 Digestibility and intake of silage

In previous experiments (Chapters 3-5) the fermentation characteristics of plantain silage in mini silos has been questionable with high pH and low lactic acid. Concern was expressed that poor fermentation characteristics would reduce acceptability, and therefore intake, of plantain silage to livestock. In this study the intake of ryegrass (1.1 kg/day) and plantain 4L silage (1.2 kg/day) of sheep was similar to that of fresh grass and plantain that accounted for 1.03 kg/day fresh grass leaves and 1.44 kg/day fresh plantain leaves, respectively (Fraser & Rowarth, 1996). These results suggested that lambs did not have trouble consuming the plantain silage from a fermentation point of view. Indeed the highest and most consistent intake was on 4L plantain, which was higher than the control. The reason for higher intake of plantain 4L compared to other plantain silages might be due to the sweet aroma of sorbitol contained in the silage which could reach more than

40% of the total WSC (Jiang *et al*. 2019), since sweet taste might increase animal preferences as stated by Goatcher & Church (1970). Furthermore, plantain 4L had higher moisture content (>60%) than that of other plantain silages (<50%) as the preference of sheep is for wet forage (Kenney & Black, 1984). The low palatability of plantain 6L and plantain 6L+mol was probably due to the higher proportion of stem in these both silages and poor digestibility leading to longer rumen retention times. Adding molasses to 6L silage improved digestibility but did not improve intake, perhaps because the gains were too small or there were other factors causing an aversion and preventing higher intake e.g. ammonia levels.

The higher digestibility of the 4L and ryegrass encouraged greater intake by sheep. In this study, the DM intake of plantain 4L silage was as high as ryegrass silage. The reason for the high intake of ryegrass and plantain 4L was because these silages contained high leaf proportion which was highly digestible. This is also evidenced by the high proportion of stem rejected by sheep in feed refusal, accounting to more than 50% (Table 6.6). This agrees with Baumont *et al*. (2000) who stated that sheep prefer highly digestible feed and the feed which can be ingested quickly and rapidly. Furthermore, (Provenza *et al*. 2007) reported that sheep prefer feeds richer in energy but will still choose some straw to prevent rumen disorder (Cooper, Clough, & Clark, 2000).

This study focused on digestibility as digestibility is the major feed factor that influenced feed quality, then, in turn, affects animal performance (Keady, Hanrahan, Marley, & Scollan, 2013). Figure 6.3 clearly highlights the relationship between in vivo digestibility and animal performance, which is reflected by sheep weight gain (P=0.001). Compared with traditional ryegrass silage, plantain silage was poorer quality irrespective of harvest time and additive. The superior quality of ryegrass is caused by the high portion of leaves (90%) which are highly digestible in ryegrass, whereas the leaf percentage of plantain 4L silage was low (less than 35%). The digestibility of plantain 6L silage was not high and it could not be increased by using molasses. The possible reason is because of the high proportion of the mature stem (>50%) as the mature stem has low digestibility (Lee *et al*. 2015). Nevertheless, the digestibility of plantain at the late-harvest in this study, which had a value of around 60%, was not inferior compared with the digestibility of plantain silage found by Raeside *et al*. (2012) who reported that plantain digestibility at the late-harvested was accounted for 56%.

Comparing with fresh material, the digestibility of ryegrass was not too different. The decrease was about 4%. However, plantain experienced a more substantial decline in digestibility, which decreased by 10%. The reduction of digestibility might be because of the decrease in the digestible

material during the ensiling process (Buckmaster, Rotz, & Muck, 1989), where the digestible part of silage was being used by microorganisms in silage for their energy (Gibbs *et al*. 1950).

Animal performance is closely related to the digestibility of feed. High digestibility led to an increase in weight gain of animals, and conversely, low digestibility causes a decrease in animal body weight. It can be noted in this study that plantain 6L and even molasses treated plantain 6L caused weight loss in sheep since the digestibility of these type of silages were only below 60%. Loss of weight gain of 106 g/day from lambs fed plantain silage at the late harvested was also reported by Raeside *et al*. (2012). The weight loss of lambs fed plantain 6L and plantain 6L treated molasses in this study (Table 6.7) was likely because of the low ME intake of animals fed these silages. The feed ME intake did not meet the ME maintenance requirements of these lambs, where the ME maintenance requirement should have be 7.3 MJ ME per day (Table 6.2.), but the ME intake of plantain 6L was 7.3 and plantain 6L+mol was 6.9 MJ ME/kg DM (Table 6.7.) so lambs needed to eat at least a kilogram of plantain to maintain liveweight – let alone support liveweight gain.

6.4.2 Nitrogen utilisation

Our measurements were unable to explain the destination of all consumed N, as reflected by N balance. Incomplete balance is commonly observed in a number of feeding trials and the causes are not always clear. We acknowledge that while every attempt was made to collect and measure everything the estimation of nitrogen balance in this study may not be accurate. The source of the inaccuracy of nitrogen balance could be from main sources (Spanghero & Kowalski, 1997). The first is incomplete collection faecal material and ammonia losses from faeces in stall and or during sample drying, the second might be from volatile N losses during urine collection, and the third is by the loss of dermal and scurf losses of the sheep. Alternatively, the adaptation period in our study was relatively short and there may have been a labile pool of body N which biased total N collection (Hristov *et al*. 2019). The incomplete N balance is a major limitation of this study, making it difficult to draw conclusions regarding N utilisation.

It appears from our results that feeding plantain silage improves N utilisation, though at the expense of productivity as the plantain silages reduced intake and liveweight gain compared with the control Although improved nitrogen utilisation in fresh plantain has previously been reported (Box *et al*. 2017), nitrogen utilisation in plantain silage has not been studied before.

It has been suggested that secondary metabolites in fresh plantain may be involved in diuresis in livestock consuming fresh plantain (O'Connell *et al*. 2016) to reduce urinary N load (Box *et al*. 2017; Navarrete *et al*. 2016). However, we found that sheep fed plantain silage did not show diuresis as urine volumes were similar for treatments. This may be due to the absence of secondary compounds in the silage or other changes relating to moisture content.

Determining the role of secondary compounds in fresh or ensiled forage on animals was beyond the scope of this study, other than to report that there are evidently alterations in secondary compounds during ensiling. However, because of the need for farmers to improve environmental practises, it was the purpose of this research to ascertain whether feeding plantain silage could reduce urinary N excretion, either through lower N intake or by other means. It is unlikely that secondary compounds were responsible for the reduced N excretion observed in this study. The reduced nitrogen excretion pattern in sheep fed plantain 4L because of lower CP intake. A close relationship between protein or N intake and N excretion has been demonstrated previously (Kebreab, France, Mills, Allison, & Dijkstra, 2002).

6.5 Conclusions

The results presented here have demonstrated the potential of plantain silage at the early regrowth stage for ruminants. Besides, from an environmental view, plantain was best for mitigating nitrogen leaching to the soil because the ratio between urinary nitrogen and faecal nitrogen was lower compared with ryegrass silage. The lower nutritive value of plantain silage than conventional ryegrass silage can be compensated by supplemental feed. The utilisation of molasses as additive was useless as molasses failed to increased digestibility of plantain at the late harvested silage although molasses decreased ADF content in plantain 6L. Future work should examine the best ratio of plantain silage when combined with other feeds. However, in this study the null hypothesis was rejected because plantain silage was inferior to ryegrass silage as a control in terms of the quality and the in vivo digestibility.

Chapter 7

General Discussion

7.1 Summary and justification of this study

Ensiled forage, either as pit silage or baleage dominates forage conservation practises in New Zealand farming systems. Recently farmers are advised that growing and feeding plantain (*Plantago lanceolata*) will help to improve environmental outcomes regarding N leaching. While support exists for the establishment (Bryant *et al*. 2019) and management (Box *et al*. 2017; Cranston, Kenyon, Morris, & Kemp, 2015) of plantain into farm systems, there is little information to date on whether or how plantain might be conserved during feed surplus. Nor whether the beneficial environmental properties from the fresh forage can be retained in the ensiled forage. This thesis reports a series of experiments conducting both in vitro and in vivo trials investigating pre and post harvest management of plantain on ensiling properties.

Briefly, the first experiment (Chapter 3) evaluated the quality of plantain silage at different regrowth stages, and storage durations, made from spring harvest, while the second experiment repeated the first for autumn-harvested plantain (Chapter 4). The third experiment (Chapter 5) evaluates the quality of plantain silage with different fertilizers and feed additives of mature plantain harvested in spring. Finally the last experiment (Chapter 6) brought together findings from previous studies in an attempt to evaluate animal response to commercial scale plantain silage. The general discussion provides an integrated discussion of the main findings in the research chapters and further work.

7.2 The main finding of this research

This study focussed on plantain silage management as a strategy to improve fermentation characteristics and enhance nutritive value for optimal animal response to silage as a supplement. The first three experiments found that plantain silage had generally poor fermentation characteristics and low nutritive value based on *in vitro* digestibility and protein content. Not surprisingly factors which affect leafiness of the forage pre harvest affected nutritive value. For example, autumn harvest or 4L harvest in spring improved in vitro digestibility. Using enzyme or bacterial additives did little to improve fermentation characteristics, though pre harvest

management and storage duration had some influence on fermentation of plantain silage. N fertilizer and molasses improved the fermentative characteristics of plantain silage at the late regrowth stage without any improvement in *in vitro* digestibility. In spite of apparently poor fermentation and low nutritive value, the lambs to which these silages were fed, consumed the plantain silages readily, indicating that plantain silage is safe to feed to livestock. The silages had low protein content and reduced N losses, though low digestibility resulted in poor animal performance.

7.3 Fermentation characteristics of plantain silage

Overall, the fermentation characteristics of plantain silage were not satisfactory. However, ensiling plantain at the time of the leafy plantain stage was optimal to improve fermentation. For spring, the best time for ensiling was at the early stage of maturity while, in autumn, the best time for ensiling was at the late stage of maturity. The autumn results highlighted that it should not be a target to ensile a forage which is too leafy as the quality of autumn silage might be decreased with the production of effluent from the moisture in the silage during autumn is a risk because of very low dry matter.

Plantain is a prolific seed producing plant over late spring and summer so the quality of the silage crop was anticipated to be quite low. The hypothesis that using additives would improve plantain silage was not accepted as utilisation of additives in plantain made no benefits. Molasses used in this study just improved the fermentation characteristics of plantain silage without any improvement in the digestibility. The cost and benefits of adding molasses needs to be considered because the additional value of molasses to silage quality may not outweigh the costs.

Fermentation characteristics of silage between the mini silo and commercial silo were similar adding confidence to our conclusions from the mini-silo work. Although in the mini silo's it was more difficult to exclude oxygen by compression and oxygen ingress through the plastic – so the drop in quality was greater in the mini-silo than in the large bale. Plantain silage easily became mouldy, especially during the feed out period when using the baleage which we fed to sheep over a number of days. From a practical perspective, our results support ensiling plantain as baleage can be used to feed to the animal quickly compared to pit silo which is at risk of becoming mouldy due to poor stability of plantain silage.

Another possibility to improve fermentation characteristics is by chopping plantain before ensiling. This option was considered at the beginning of this research program and a pilot study was carried out using plantain of varying chop lengths in mini-silos. In our pilot study there was no difference in silage fermentation characteristics between chopped and unchopped plantain in the mini silo. Hence, we opted to pursue effect of management on unchopped plantain as most silage contractors do not chop pasture silage prior to ensiling.

All of these studies showed what seemed to be poor fermentation characteristics in plantain silage. The poor fermentation characteristics of plantain silage were because of the high pH (>5.0) and high buffering capacity (BC >200 meq) of plantain. Usually, the pH of silage is affected by the buffering capacity of the original crop (Kung Jr, 2010). For example, the two different crop silages may have similar pH, but they may have varied BC, such as lucerne that has a higher buffering capacity than that of corn making lucerne the more difficult to ensile (Playne & McDonald, 1966). A plant having high pH and BC will have high pH and BC when they are ensiled. We carried out some retrospective assessment of plantain and ryegrass pasture which supported this opinion. Fresh plantain at the early stage of maturity had a pH of 5.1 and BC of 113meq/100 g DM. Mature plantain had a pH of 5.8 and BC of 101 meq/100 g DM. In contrast, ryegrass at the early stage of maturity had a pH of 6.2 and low BC of 87.7 meq/100 g DM (unpublished data, 2019). There is scope for additional work in this area of BC and pH which may also be linked to microbial interactions in the soil and in the rumen as well as the silage.

7.4 Nutritive value of plantain silage

Across a range of pre and post-management treatments, the estimated energy density of plantain silage ranged from 7.05 MJ ME/kg DM to 10.4 MJ ME/kg DM. In a survey of ryegrass silage in New Zealand by Howse *et al*. (1996), silage quality ranged from less than 8 to more than 11. The silages in this study are not hugely different to the range from the silage quality of New Zealand silage. The range of silage quality in this study was largely driven by pre-ensiling management factors which affected botanical composition. The stem proportion of plantain at the late regrowth stage in spring was greater compared to plantain at the early regrowth stage. Nevertheless, plantain produced in Autumn had the greatest quality as there was no stem.

The quality of silage in mini silo ranged from 6.87 MJ ME/kg DM – 10. 4 MJ ME/kg DM. The digestibility ranged from 47.9 -76 % and the protein content ranged from 11.2 – 23 %. Pre and post-harvest management had an effect on silage quality. Harvesting plantain at the early stage of

maturity was more beneficial than harvesting plantain at the late stage of maturity. This is because at the early stage of maturity was more leafy than that of at the late stage of maturity. The leafy plantain resulted in longer shelf life than that of stemmy plantain silage. In this case, plantain silage made in autumn had the greatest value regarding the nutritive value.

We were interested to understand whether ranking of silage digestibility would alter between laboratory and animal studies. Generally speaking, in vitro DMD agreed with in vivo digestibility, which is demonstrated in Figure 7.1. When *in vitro* predictions of digestibility are plotted against *in vivo* values, the *in vivo* digestibility of forage silage in this study was only higher by 4.5% than the *in vitro* digestibility estimated by NIRS. The correlation was significant, where P = 0.003. Though the R-square was poor due to large variability, this meant that in vitro digestibility using pepsin- cellulase method could be used to determine the digestibility of the feed. Besides, it is cheap, and in vitro digestibility did not take a lot of labor and time.

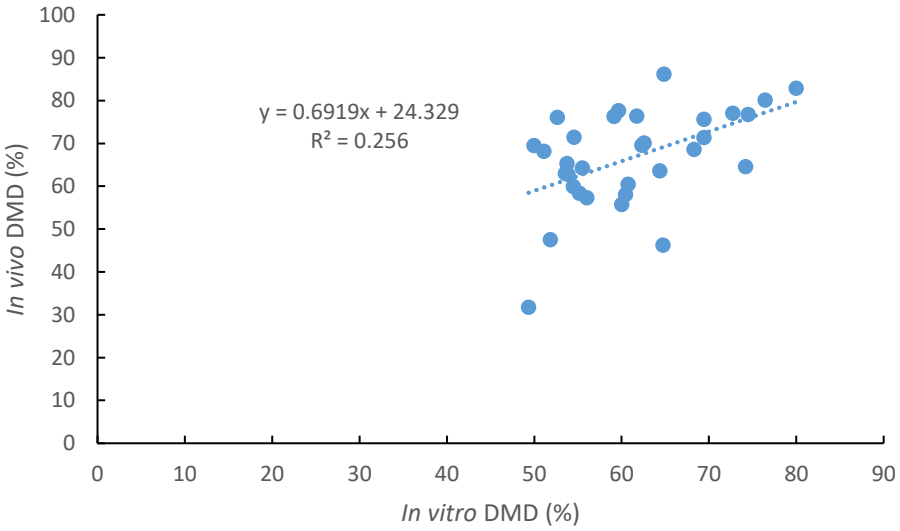

Figure 7.1. Relationship between *in vitro* and *In vivo* DM digestibility (%)

7.5 Implication for reducing N loss

Plantain silage as a tool to help mitigate N losses is likely to differ from how plantain is used as a grazed fresh forage. Nitrogen intake was still higher for ryegrass silage compared with nitrogen intake in plantain silage. Thus, the N excretion in plantain silage was lower than the nitrogen excretion pattern in ryegrass silage. From this point, the use of plantain silage as a low N supplement can provide environmental benefits. Combining results showing poor digestibility with low protein, a

farmer could use plantain silage at the late regrowth stage to supplement an animal which needs to meet maintenance requirement. At this stage, plantain silage is a good supplement for dry stock where farmers are trying to reduce N intake. But for high performing animals such as lactating or young growing animals as a supplement there is need to ensile plantain while leafy to maintain quality

7.6 Challenge for further research

7.6.1 Research at the farm level

These studies were conducted indoor, further study using outdoor with grazing animals at a farm level is recommended. Indoor compared with outdoor experiments could achieve a different result because of several factors, such as weather conditions, physiological stages of the animal. The other reason is the need to study the proportion of plantain silage in the diet of the animal for production and environmental reasons. In this experiment, the digestibility of a sole diet of plantain silage fed to sheep was still inferior compared to ryegrass silage. At the farm level a study could be designed to make a feed ration, which can include in plantain silage diet to meet the requirement of the animal.

7.6.2 Utilization of conserved plantain

Plantain autumn harvested had high digestibility (Chapter 4) compared to that in spring harvested, but the quality and palatability of this silage in baleage is still questionable. In this study, there was no chance to conduct a trial by using autumn harvested because of the limitation of the raw material of silage. It is challenging to implement plantain silage harvested in autumn on farm-scale to know the response of the animal.

Another area for future research relates to animal utilisation of silage protein and N. The results from our in vivo study demonstrated the difficulty of conducting N balance studies. We were unable to draw conclusions about N use efficiency because up to a third of the N intake was not accounted for in feces, urine or gain. Because of the low digestibility of spring or summer harvested plantain it is unlikely that this forage will make a suitable supplement to support production. But it may have value in supporting maintenance of livestock and maintain low N losses due to low protein content.

7.6.3 Secondary metabolite in plantain silage

The secondary metabolite of plantain silage needs further research as in this trial only bulk sample for analysis were used. Further research is required to understand the role of secondary metabolites

and their interactions with microbes during ensiling. It would be interesting to make silage using different plantain cultivars as different cultivars contain different proportion of secondary metabolites (Stewart, 1996).

7.6.4 Sorbitol in plantain

Finally, the sugar sorbitol is an alcohol sugar that contributes a large percentage of WSC in plantain (Jiang *et al*. 2019). The role of sorbitol in plantain silage is still questionable, although in this study, sorbitol might influence bacterial species abundance, favouring bacteria which can utilise sorbitol, this could potentially alter the fermentation products. Further knowledge about sorbitol will be worthy of plantain silage. In this study, there was no chance to investigate sorbitol in plantain silage as sorbitol analysis is costly.

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